INDIAN AGRICULTURAL RESEARCH INSTITUTE (PUSA)
IARI
जीव-जीवनकृषिः

LIBRARY
New Delhi

CEREAL CHEMISTRY

Published by the American Association of Cereal Chemists

Editor in Chief - - - W. F. GEDDES
Assistant Editor - - - KATHLEEN WEBB
Managing Editor - - - DONALD E. SMITH

Editorial Board:

J. A. ANDERSON
J. S. ANDREWS
M. J. BLISH
G. GARNATZ
BETTY SULLIVAN
C. O. SWANSON

II—

VOLUME XXIII · Nos. 1-6
JANUARY NOVEMBER, 1946

Lancaster, Pennsylvania
1946

CONTENTS

NUMBER 1, JANUARY, 1946

Methods for Determination of Alpha-Amylase. II. Liquefying and Dextrinizing Activities of Alpha-Amylases from Different Sources. *Sutton Redfern and Quick Landis* 1

Supercentrifugates from Dough. *J. C. Baker, H. K. Parker, and M. D. Mize* 16

The Distribution of Water in Dough. *J. C. Baker, H. K. Parker, and M. D. Mize* 30

Gas Occlusion During Dough Mixing. *J. C. Baker and M. D. Mize* 39

Effect of Leaf and Stem Rust on the Phosphorus Metabolism of Hard Red Spring Wheat. *A. Hoffer, W. F. Geddes, and M. N. Levine* 52

Rapid Determination of Bromate in Flour. *A. A. Towner and R. C. Koehn* 64

A Rapid Method for the Determination of Bromate in Bromated Flours. *A. Hoffer and A. W. Alcock* 66

The Distribution of Thiamine in Wheat Seedlings at Different Stages of Germination in the Dark. *A. Hoffer, A. W. Alcock, and W. F. Geddes* 76

Further Studies on the Mechanism of the Action of Oxidation and Reduction on Flour. *Marjorie Howe* 84

Analyses of Double-Cross Hybrid Corn Varieties Produced on Farms. *J. J. Curtis and F. R. Earle* 88

Loaf Volume Potentialities, Buffering Capacity, and Other Baking Properties of Soy Flour in Blends with Spring Wheat Flour. *Karl F. Finney* 96

Effect of Yeast, Bromate, and Fermentation on Bread Containing Soy Flour. *E. G. Bayfield and Emery C. Swanson* 104

Book Review. *Methods of Analysis of the American Society of Brewing Chemists.* Prepared by Technical Committee and Editorial Board, Louis Ehrenfeld, Editor 113

Book Review. *Physical Methods of Organic Chemistry. I.* Edited by Arnold Weissberger 114

NUMBER 2, MARCH, 1946

A Machine for Measuring the Extensibility and Resistance to Extension of Gluten. *K. Hlynka and J. Ansel Anderson* 115

Essential Amino Acid Composition of Feed Materials. *Werner Baumgarten, Adaline N. Mather, and Leonard Stone* 135

Effect of Method of Combining the Ingredients upon the Quality of the Finished Cake. *Gwendolyn L. Tinklin and Gladys E. Vail* 155
Study of Some Factors Influencing the Oxidation of Thiamine to Thiochrome. *H. A. Watson* 166
The Phosphorylase of Waxy Maize. *Laura Bliss and Nellie M. Naylor* 177
Some Effects of Cabinet Fermentation on Sponge Temperatures and Dough and Bread Characteristics. *Floyd Schoonover, Jacob Freilich, and Sutton Redfern* 186
Amino Acids in Corn Grain from Several Single Cross Hybrids. *D. M. Doty, Merlin S. Bergdoll, Harold A. Nash, and A. M. Brunson* 199
An Automatic Device for the Measurement of Gas Production and Gas Retention in Doughs. *Earl B. Working and Emery C. Swanson* 210
Effect of Flour Lipids on Recovery of Gluten from Hard and Soft Wheat Flours by the Use of Sulfite Solutions. *Irwin W. Tucker* 217

Number 3, May, 1946

Grain Storage Studies. III. The Relation Between Moisture Content, Mold Growth, and Respiration of Soybeans. *Max Milner and W. F. Geddes* 225
A Study of the Mill Streams Composing 80% Extraction Flour with Particular Reference to Their Nutrient Content. *C. R. Jones and T. Moran* 248
Use of Spray-Dried Whole-Egg Powder in Sponge Cakes. *Ruth Jordan and Margaret S. Pettijohn* 265
Chemical State of Phosphorus in Starch as Indicated by Titration Curves on Electrodialyzed Starches. *D. R. Briggs and Martin Hanig* 277
Preparation and Nitrogen Content of Soybean Protein. *William G. Smiley and Allan K. Smith* 288
Staling Studies on Breads Containing Waxy Maize Starch. *P. P. Noznick, P. P. Merritt, and W. F. Geddes* 297
Effect of Soy Flour and Nonfat Dry Milk Solids in White Bread on the Nutritional Quality of the Protein as Measured by Three Biological Methods. *Shirley C. Carlson, F. H. Hafner, and J. W. Hayward* 305
The Vitamin B-Complex Content of the Commercial Milling Products of Egyptian Rice. *Stanley A. Price* 318

The Quantitative Determination of Molds in Flour. *Clyde M. Christensen* .. . 322
Relationship of Zein to the Total Protein in Corn. *D. W. Hansen, B. Brimhall, and G. F. Sprague* 329
Book Review. *Frontiers in Chemistry. IV. Major Instruments and Their Applications to Chemistry.* Edited by R. E. Burk and Oliver Grummitt 335
Book Review. *Organic Preparations.* By Conrad Weygand..... 336

Number 4, July, 1946

Photomicrographic Studies of Wheat Starch. I. Development of the Starch Granules. *R. M. Sandstedt* 337
The Proteolytic Enzyme Activity of Milled Fractions of Wheat and the Effect of Bromate in Flour. *Marjorie Howe and David Glick* 360
Open Trough and Cabinet Fermentation of Bread Sponges. *Betty Sullivan and W. A. Richards* 365
Farinograms and Mixograms as a Means of Evaluating Flours for Specific Uses. *John A. Johnson, J. A. Shellenberger, and C. O. Swanson* 388
Extensograph Studies of Commercial Flours and Their Relation to Certain Other Physical Dough Tests. *John A. Johnson, J. A. Shellenberger, and C. O. Swanson* 400
Amylograph Curve Characteristics of Various Types of Commercial Flours and Their Relation to Flour Maltose and Gassing Power Values. *John A. Johnson, J. A. Shellenberger, and C. O. Swanson* 410
Water Retention Capacity as an Index of the Loaf Volume Potentialities and Protein Quality of Hard Red Winter Wheats. *Karl F. Finney and William T. Yamazaki* 416
Composition of Hybrid Corn Tassels. *J. M. Van Lanen, F. W. Tanner, Jr., and Shirley E. Pfeiffer* 428
Gas Production and Gas Retention of Dough as Affected by Type of Flour, Baking Formula, and Amount of Mixing. *Emery C. Swanson and C. O. Swanson* 432
Report of the 1945-46 Committee on Testing Biscuit and Cracker Flours. *Frank R. Schwain* 440
Book Review. *Studies on the Nature of the Bromate Effect.* By Holger Jørgensen 446

Number 5, September, 1946

Grain Storage Studies. IV. Biological and Chemical Factors Involved in the Spontaneous Heating of Soybeans. *Max Milner and W. F. Geddes* 449

The Effect of Pressure on Macaroni Discs: Size and Number of Air Bubbles in Relation to Light Transmission. *Glenn S. Smith, R. H. Harris, Ethel Jesperson, and L. D. Sibbitt* 471

A Micro-Milling Technique Using the Hobart Grinder. *Karl F. Finney and William T. Yamazaki* 484

A Study of Sorghum and Sorghum Starches. *F. E. Horan and Marie F. Heider* 492

Composition of the Component Parts of the Corn Kernel. *F. R. Earle, J. J. Curtis, and J. E. Hubbard* 504

Influence of Ingredients on Thiamine Retention and Quality in Baking Powder Biscuits. *Alice M. Briant and Marcia R.* Hutchins. 512

Report of the 1945 46 Methods of Analysis Subcommittee on Thiamine Assay. *Aaron Arnold* 521

Book Review. *Hackh's Chemical Dictionary.* By Julius Grant 527

Book Review. *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.* Published by the A.O.A.C. 527

Book Review. *The Baking Industry.* By Mary Kinnavey Moore 528

NUMBER 6, NOVEMBER, 1946

Effect of Parboiling on Thiamine in Rice. *M. C. Kik* 529

Studies of the Composition of the Wheat Kernel. III. Distribution of Ash and Protein in Central and Peripheral Zones of Whole Kernels. *V. H. Morris, Thelma L. Alexander, and Elizabeth D. Pascoe* 540

Photomicrographic Studies of Wheat Starch. II. Amylolytic Enzymes and the Amylase Inhibitor of the Developing Wheat Kernel. *R. M. Sandstedt and O. C. Beckord* . . 548

Some Observations on the Visual Color of Tubular and Disc Macaroni. *L. D. Sibbitt and R. H. Harris* 559

Note on a Semimicro Milling Procedure for Durum Wheat. *M. H. Fisher and W. O. S. Meredith* 565

A Micro Method for Making Macaroni. *F. G. Martin, G. N. Irvine, and J. Ansel Anderson* 568

Effect of Gassing Rate on Loaf Volume. *H. Miller, J. Edgar, and A. G. O. Whiteside* 579

Analysis of Data for A.A.C.C. Check Sample Service. II. Protein and Thiamine Results, 1944–45. *W. O. S. Meredith* 585

Modification of the Gas Production and Gas Retention Properties of Dough by Some Surface Active Reducing and Oxidizing Agents. *Emery C. Swanson and C. O. Swanson* 590

Determination of Urea and Ammoniacal Nitrogen in Feeds. *Margaret Schmoll and Frederick D. Schmalz*.............. .. 600
Book Review. *Physical Methods of Organic Chemistry. II.* Edited by Arnold Weissberger.. 602
Book Review. *Enzymes and Their Role in Wheat Technology.* Edited by J. A. Anderson............................... 602
Book Review. *Currents in Biochemical Research.* Edited by D. E. Green.. 603
Book Review. *The Chemistry of the Carbon Compounds. Volume III. The Aromatic Compounds.* By Victor von Richter, edited by Richard Anschütz............................ 604
Author Index.. 606
Subject Index.. 609

CEREAL CHEMISTRY

VOL. XXIII JANUARY, 1946 No. 1

METHODS FOR DETERMINATION OF ALPHA-AMYLASE. II. LIQUEFYING AND DEXTRINIZING ACTIVITIES OF ALPHA-AMYLASES FROM DIFFERENT SOURCES

SUTTON REDFERN and QUICK LANDIS [1]

The Fleischmann Laboratories, Standard Brands, Inc., New York, N. Y.

(Read at the Annual Meeting, May 1944; received for publication July 27, 1945)

Three general methods have been used in the study of amylases, viz., saccharification, dextrinization, and liquefaction. It has been amply demonstrated that saccharification methods measure a summation of the effects of alpha- and beta-amylase. Dextrinization methods based on the original method of Wohlgemuth (1908) also measure the combined effect of alpha- and beta-amylase as shown by Sandstedt, Kneen, and Blish (1939) and Hanes and Cattle (1938). No methods are available for determining the amount of beta-amylase without correction for any alpha-amylase that may be present concomitantly. There are, however, two methods at present available for specifically determining alpha-amylase: the measurement of the dextrinization time in the presence of an excess of beta-amylase and the liquefaction procedures of Jozsa and Johnston (1935) and Blom and Bak (1938).

Hollenbeck and Blish (1941) presented data which showed satisfactory parallelism of the effects of temperature changes on liquefying and dextrinizing activities for three types of alpha-amylases: malt, bacterial, and fungal. Dickson (1943) also found from a collaborative study on a series of malts that there was a high correlation between the liquefying and alpha-dextrinizing methods, indicating that these methods were measuring the same thing. Dickson's work was done on only one type of alpha-amylase, viz., malt. All of Hollenbeck and Blish's experiments except one were done by inactivation studies of individual amylases. Thus, if 50% of the enzyme as measured by the liquefying method was inactivated, then approximately 50% was inactivated as measured by the dextrinizing method.

---

[1] Deceased.

These authors went one step further, however, and stated that when extracts of the three enzymes were adjusted to an equal basis in terms of alpha-dextrinizing power, their liquefying activities also were found to be substantially the same. Only one experiment to test this was given and the outflow times of the starch pastes which should have been equal did not agree too well.

The present paper resulted from attempts to intercompare alpha-amylases from different sources by the two methods. Certain discrepancies were noted which it was felt required further investigation before the methods could be properly standardized according to the principles developed by Landis (1945).

Types of Enzymes Used

Three types of amylases were selected for study, those of barley malt, bacteria, and mold. The different enzyme preparations studied and their sources are described in Table I.

TABLE I

DESCRIPTION OF ENZYMES

Enzyme	Source	Commercial name
Malt	Barley malt	Dry distillers malt
Malt syrup A	Barley malt	T.P. Diastafor
Malt syrup B	Barley malt	Heavy Diastafor
Malt syrup C	Barley malt	Special Diamalt
Malt syrup D	Barley malt	Regular Diamalt
Takadiastase A	Mold	Takadiastase
Takadiastase B	Mold	Takadiastase
Fungal A	Mold	Clarase 700
Fungal B	Mold	Rhozyme
Fungal C	Mold	Maltase Prepn. No. 20
Bacterial A	Bacteria	Laboratory Prepn.
Bacterial B	Bacteria	Rapidase, conc.

Methods

Liquefying Activity. The viscometric method developed by Landis (1943) was used for the determination of the liquefying activity. This method is considerably more convenient to use than the regular Jozsa and Johnston method and permits one to run many more samples in one day. The details of the method will be given in the following paper of this series. In brief, the method consists of determining the percentage liquefaction produced by the enzyme acting on a special starch substrate. A constant time of one hour, a pH of 5.2, and a temperature of 30°C was used for all the dilution curve work.

The enzyme solutions were prepared with 0.025 molar calcium chloride solution.

Dextrinizing Activity. Three methods were used. The regular method as described by Sandstedt, Kneen, and Blish (1939) was used to determine the relative alpha-dextrinizing (SKB) activities of the different enzymes, and the same method without addition of excess beta-amylase to determine the relative Wohlgemuth activities.

In order to be able to study the kinetics of the dextrinization reaction, a colorimetric procedure devised by Landis (1943) was used. The substrate was prepared according to the directions of Sandstedt, Kneen, and Blish. An inorganic color standard which was found to compare closely with the iodine-dextrin solution of these authors was used for color comparisons. It consisted of 20 g $CoCl_2.6H_2O$, 3 g $K_2Cr_2O_7$, and 10 ml of 0.1 *N* HCl made up to 100 ml with distilled water. A Klett-Duboscq colorimeter was used. The proportions of enzyme infusion to substrate solution were as specified in the method of Sandstedt *et al.* At appropriate time intervals, 2 ml of the reacting mixture were added to 10 ml of iodine solution "B" and color comparisons were made. A Wratten "A" red filter was placed in the eyepiece of the colorimeter in order to improve the ease of matching without having to take into account the color changes taking place in addition to intensity changes. The standard solution was used at a depth of 20 mm. The unknown colorimeter readings expressed in millimeters were used directly as a measure of the amount of substrate changed.

The enzyme solutions for the dextrinizing method were also prepared with 0.025 molar calcium chloride solution.

Intercomparison of the Liquefying and Dextrinizing Strengths of the Different Amylase Preparations

In order to put the liquefying determinations on a basis which does not depend on calibration or initial rates and is independent of any enzyme units, the method of "equivalence by superposition" (Landis, 1945) is used. By this method, the amount of each enzyme preparation required to produce a given amount of conversion in a given time is determined. However, in order to make it practical, different concentrations of the enzyme were used and the amount of conversion in constant time is determined. By interpolation, the amount of enzyme to give any intermediate degree of conversion is easily determined and the results may be checked at several points by this technique.

The dilution curves determined for each of the three types of alpha-amylases are shown in Figure 1. For the malt and bacterial amylases the curves are essentially parallel straight lines over the dilution range studied. The takadiastase dilution curve is markedly different. The ratio of the enzyme strengths of these three preparations may be calculated, from the weight of each enzyme required, to give any degree of conversion as read from the curve. The ratio may also be calculated graphically by measuring the distance between the curves since the sample weights are plotted on a logarithmic scale.

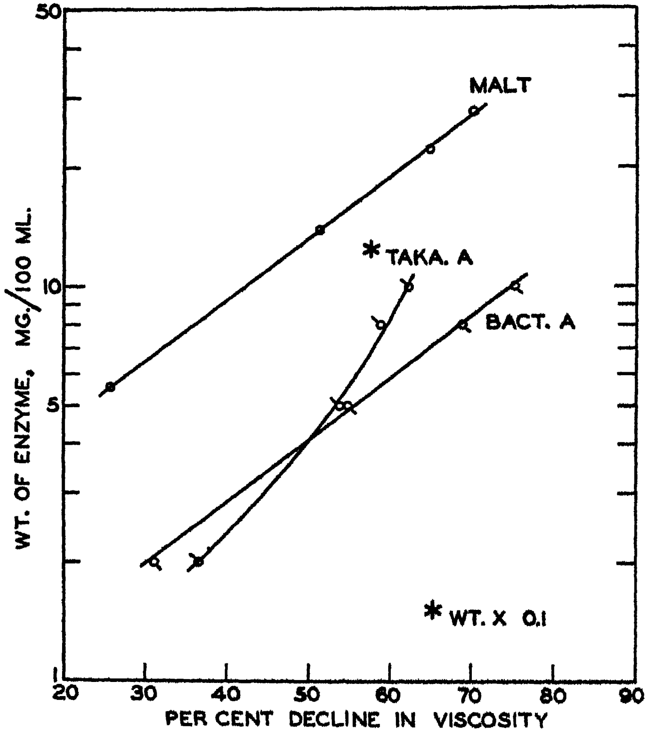


Fig. 1. Liquefaction-dilution curves for different alpha amylases.

This method of plotting makes it possible to determine by mere inspection whether the enzymes can be compared at different degrees of conversion since the curves will be parallel if this is true. Since the enzyme strengths are inversely proportional to the equivalent weights, it is easy to calculate the strength ratios, assuming the malt to have a value of unity at any given percentage decline in viscosity. These values are shown in Table II.

Table II shows that the malt and bacterial enzymes may be compared at any degree of conversion since the dilution curves are

TABLE II

COMPARISON OF RELATIVE STRENGTHS FOR DIFFERENT ENZYMES

Enzyme	Ratio of strengths						
	Percentage decline in viscosity						SKB[1]
	40	45	50	55	60	70	
Malt	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bacterial A	3.24	3.24	3.24	3.22	3.22	3.22	2.28
Takadiastase A	39.2	36.3	33.0	28.4	23.1	—	29.9

[1] Refers to the Sandstedt, Kneen, and Blish method.

parallel. The takadiastase varies and when compared to malt its activity varies almost twofold depending upon the degree of conversion selected for comparison.

The relative strengths of these three enzymes as determined by the alpha-dextrinizing method are also shown in Table II. It is to be noted that the three preparations definitely show different relative strengths when compared by the two methods. This is contradictory to Hollenbeck and Blish's work.

It might be questioned that these results were peculiar to the three samples of alpha-amylases used. That this is not so is shown in Figure 2, where the dilution curves of a malt syrup, another bacterial preparation, and three different fungal amylases are compared. One of the fungal enzymes, sample C, was developed for a high maltase activity, but this did not significantly alter the curve. Again we find that the bacterial and malt amylase dilution curves are essentially parallel, while the fungal enzyme curves are different, although they are all roughly parallel to one another.

It must be noted that one set of curves cannot be compared exactly with another set. Each set represents determinations made on one batch of substrate in order to eliminate the question of unavoidable daily differences caused by the inability to duplicate the initial viscosity of the substrate accurately on different days.

Since the three types of alpha-amylases show different strength ratios by the two methods, it is difficult to understand how Hollenbeck and Blish found equivalent outflow times for a starch paste liquefied by equivalent alpha-dextrinizing concentrations for a constant time. Nevertheless, a further check of this point was made. A careful analysis of samples of the three enzymes gave relative alpha-dextrinizing and liquefying ratios as shown in Table III.

Using weights of each enzyme inversely proportional to the alpha-dextrinizing ratios, liquefying rate curves were run, as shown in

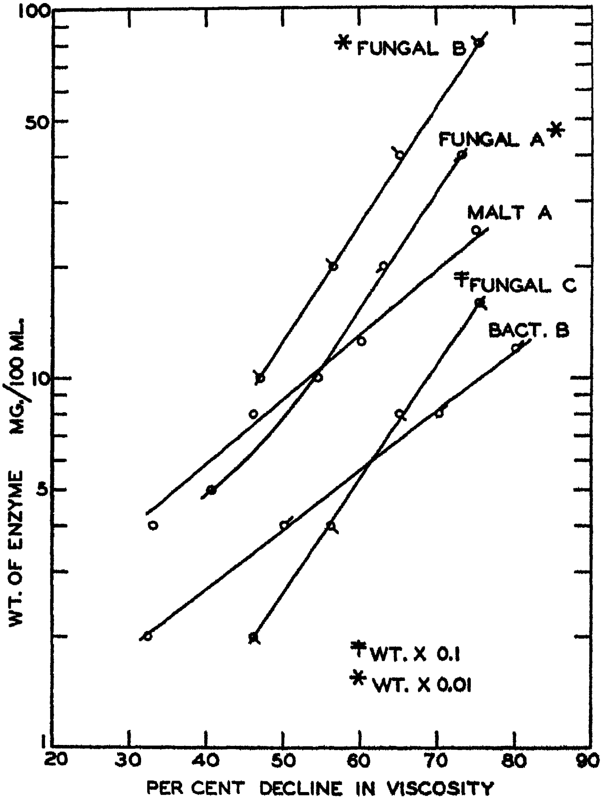


Fig. 2. Liquefaction-dilution curves for different alpha-amylases.

TABLE III

RELATIVE ENZYME STRENGTHS USED FOR RATE CURVES

Enzyme	Relative strengths by	
	Liquefaction	Alpha-dextrinization
Malt syrup A	1.0	1.0
Bacterial A	2.03	1.28
Takadiastase A	23.6[1]	19.4

[1] Equivalent initial rate.

Figure 3. The curves are not superimposable, which they should be if the two types of activities are equivalent. The bacterial amylase is evidently more active in the liquefying method than in the dextrinizing method. The takadiastase is quite different. It produces a rapid initial decrease in viscosity, then the rate falls off. The experimental results seem to indicate that the results of Hollenbeck and Blish were

perhaps fortuitous. Calculations from their data show that the final outflow times are equivalent to a viscosity decline of approximately 92%. At this point the viscosity change is rather slow and any real differences may have been masked.

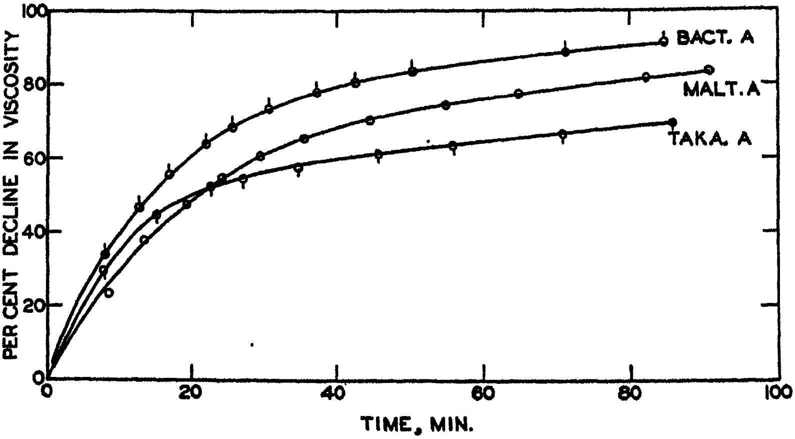


Fig. 3. Liquefaction rate curves for equivalent alpha-dextrinizing quantities of different alpha-amylases (malt A, 25.0 mg; bacterial A, 19.5 mg; takadiastase A, 1.29 mg; all per 100 ml).

Liquefying rate curves for equivalent liquefying quantities of the enzymes were also studied, with the same quantity of malt syrup used above as a basis. In this case an amount of takadiastase was chosen to give an initial rate approximately equal to the other two. Figure 4

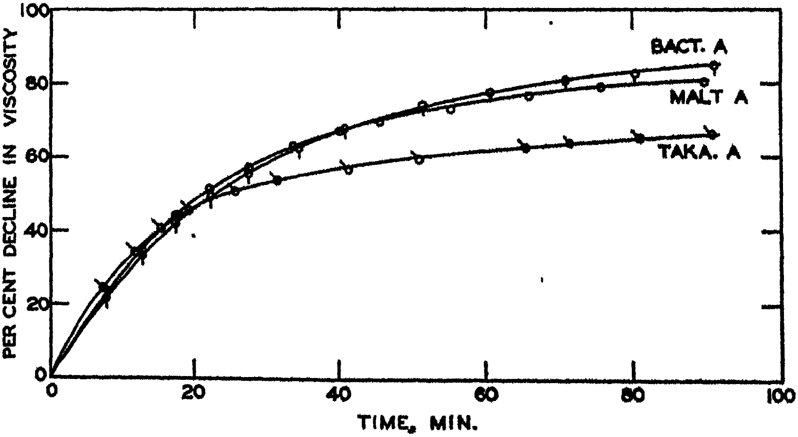


Fig. 4. Liquefaction rate curves for equivalent liquefying quantities of different alpha amylases (malt A, 25.0 mg; bacterial A, 12.3 mg; takadiastase A, 1.06 mg; all per 100 ml).

shows that the malt and bacterial amylases give nearly equivalent rate curves, but again the takadiastase exhibits an entirely different kinetic picture. The rate very quickly falls off and the enzyme action slows down markedly so that the amount of work done in the longer times is much less than would be calculated from the initial rate.

A similar dilution curve study was made for the dextrinizing method using Landis' colorimetric modification. The ordinary method of Sandstedt, Kneen, and Blish gives a unique answer when different enzymes are compared, but yet if they were compared at some other end-point than the one chosen by these authors, a different result might be obtained, as has already been demonstrated for the liquefying method. The same general procedure was used for the colorimetric method as for the liquefying method. The colors produced by varying quantities of enzyme in constant time were determined and the dilution curve plotted on log-log coordinate paper. These scales were chosen merely to give approximately straight lines. Exactly the same reasoning as in the liquefying method was used. At equal colors in constant time the enzymic strengths are in the inverse ratio of the weights required.

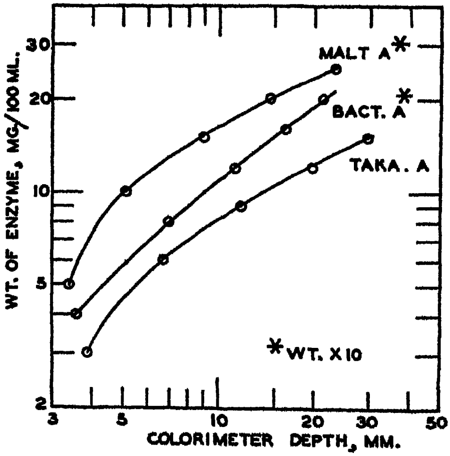


Fig 5. Colorimetric alpha-dextrinizing dilution curves for different alpha-amylases.

Figure 5 shows the colorimetric dilution curves for the three enzymes. Again, we find differences between the three types of enzymes. In this case, however, the malt and fungal enzyme curves are nearly parallel and the bacterial one differs from the other two. It may be noted that a colorimeter reading of 20 mm corresponds approximately to the regular dextrin-iodine end-point color. Table IV gives the relative strengths obtained from Figure 5.

TABLE IV

RELATIVE STRENGTHS OF DIFFERENT ENZYMES AT DIFFERENT COLOR END-POINTS

Enzyme	Relative strengths							
	Colorimeter readings, mm							
	4	6	8	10	15	20	25	Avg
Malt syrup A	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bacterial A	2.0[1]	1.72	1.61	1.52	1.37	1.28	1.20	1.45
Takadiastase A	27.0[1]	23.0	21.1	20.1	19.0	18.5	18.0	19.9

[1] Not included in average.

The ratios obtained at a reading of 20 are, within the experimental error, the same as the ones already obtained by the regular dextrinizing method. At other colors the fungal enzyme gives approximately the same relative strengths when compared with the malt enzyme, but the bacterial enzyme gives varying results. Therefore, the dextrinizing method also gives results which depend on the end-point.

Intracomparison of the Liquefying and Dextrinizing Strength of Different Amylase Preparations

Kneen, Sandstedt, and Hollenbeck (1943) have noted that "beta-amylase prepared from barley or wheat is applicable in the preparation of a substrate for the determination of *cereal* alpha-amylase activity, but the advisability of its use for the determination of the activity of other 'alpha-amylases,' such as those of animal or microbial origin, has not been established and is not recommended." Nevertheless, from preliminary work, it seemed that either a dextrinizing method or a liquefying method could be used for intracomparisons of the different alpha-amylases, but not intercomparisons.

Experiments to check this were made as follows. Dilution curves for four types of malt syrups were made and from these the relative activities are determined. The dilution curves are shown in Figure 6 and the ratios of their strengths are shown in Table V. The average ratios are compared with the values determined by the alpha-dextrinizing method in Table VI.

The agreement is very good when it is considered that the error of each method is approximately ± 5%. To further check the agreement, rate curves for equivalent alpha-dextrinizing quantities of enzyme were run. The agreement is so good that it is too difficult to show on a graph because the points all run together.· The maximum deviation between the curves is 1.2% decline in viscosity, which is excellent. It may be concluded that a series of malts may be intracompared by either the alpha-dextrinizing or the liquefying method.

TABLE V

COMPARISON OF LIQUEFYING METHOD FOR MALT SYRUPS

Malt syrup	Relative strengths						
	Percentage decline in viscosity						
	35	40	50	60	70	75	Avg
A	1.0	1.0	1.0	1.0	1.0	1.0	1.0
B	1.41	1.42	1.44	1.46	1.48	1.48	1.45
C	0.324	0.322	0.320	0.315	0.310	0.308	0.317
D	0.138	0.138	0.140	0.142	0.135	0.128	0.137

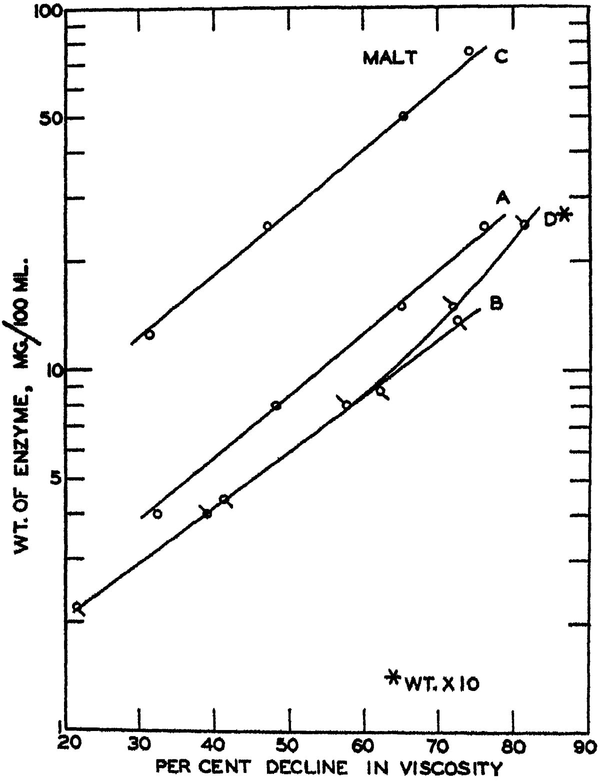


Fig 6 Liquefaction dilution curves for malt syrups

The same study was made for bacterial amylases In order to prepare samples of varying strength, some of the original material was boiled to kill the enzyme, and this boiled enzyme solution used to dilute the original material. By this means each solution varied

TABLE VI

COMPARISON OF RELATIVE STRENGTHS OF MALT SYRUPS BY LIQUEFYING AND ALPHA-DEXTRINIZING METHODS

Malt syrup	Relative strengths		
	Liquefying	SKB	Δ%[1]
A	1.0	1.0	—
B	1.45	1.42	− 2.1
C	0.317	0.271	−14.5
D	0.137	0.124	− 9.5

[1] Percentage deviation of SKB value from liquefying value.

essentially only in its enzyme content. At least all thermostable substances are retained. Three different preparations were compared by liquefying dilution curves, dextrinizing and alpha-dextrinizing activities. The results of the liquefying dilution curves are given in Figure 7 which shows their parallelism. The comparison between the methods is given in Table VII. The agreement between all three

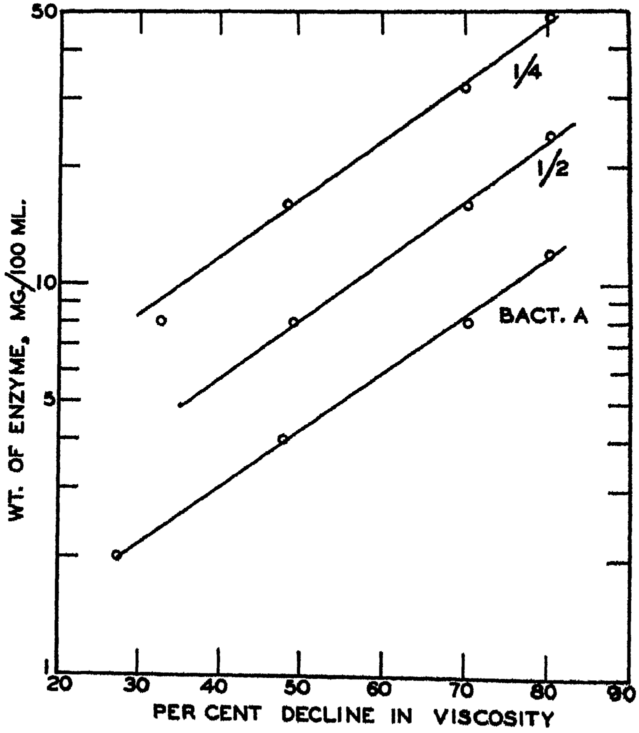


Fig. 7. Liquefaction-dilution curves for bacterial amylases.

TABLE VII

COMPARISON OF RELATIVE STRENGTHS OF BACTERIAL AMYLASES BY LIQUEFYING AND DEXTRINIZING METHODS

Bacterial amylase	Relative strengths				
	Liquefying	Wohlgemuth	SKB	Δ% Wohl.	Δ% SKB
A	1.0	1.0	1.0	—	—
A−½	0.517	0.488	0.500	−5.6	−3.3
A−¼	0.255	0.244	0.244	−4.3	−4.3

methods is excellent and it may be concluded that bacterial amylases may be intracompared by all three methods.

The same study was made of different fungal enzyme preparations. Using the values obtained from Figure 2, the relative strengths are compared in Table VIII. The agreement between the methods is quite satisfactory. The liquefaction dilution curves are not as parallel as shown for the malts or bacterials, but the deviations are not great. Any of the three methods can be used to intracompare fungal enzymes.

TABLE VIII

COMPARISON OF RELATIVE STRENGTHS OF FUNGAL ENZYMES BY LIQUEFYING AND DEXTRINIZING METHODS

Fungal amylase	Relative strengths				
	Liquefying	SKB	Wohlgemuth	Δ% SKB	Δ% Wohlgemuth
A	1.0	1.0	1.0	—	—
B	0.590	0.574	0.568	−2.7	− 3.7
C	0.287	0.272	0.324	−7.7	+12.9

Discussion

The cause of the differences between the three types of alpha-amylases is quantitative since qualitatively all cause the same general type of starch degradation. Hanes and Cattle (1938) also found quantitative differences between different types of amylases, although their work did not include bacterial amylase. Pigman (1943) has found similar differences for invertases from different sources. He found that the ratio of sucrase to inulase activity varied from 5 to 4,000. The ratio should have been constant, if all the invertases were identical. The invertases are a class of enzymes. Similarly, the amylases are a class of enzymes which differ according to their source.

It might be thought that concomitant materials may cause the differences. Some dilution curves were run, for example, on partially inactivated takadiastase, where the inactivation was done by a short heating to 50°C. The dilution curves were all parallel, although in one case the sample contained four times as much of the concomitant materials as the original sample. Another test was made by adding some boiled malt, boiled bacterial amylase, and soybean extract to a solution of takadiastase. The percent decline in viscosity was, within the experimental error, the same for all the solutions as shown in Table IX.

TABLE IX

EFFECT OF VARIOUS ADDITIONS ON THE LIQUEFYING ACTIVITY OF TAKADIASTASE

Enzyme solution	Decline in viscosity in one hour
	%
Takadiastase alone, 1.06 mg	62.9
Takadiastase + extract from 1 g soybean	63.1
Takadiastase + 250 mg boiled malt	63.0
Takadiastase + 125 mg boiled bacterial enzyme	62.5

Another cause of the differences may be union of part of the enzyme with the reaction products to form an inactive compound, or, as suggested by Hanes and Cattle, the different enzymes may have varying affinities for the lower dextrins which are produced.

A different pH is used in each of the two methods, viz., 4.7 for the dextrinizing method and 5.2 for the liquefying method. Since it is usually accepted that bacterial amylase has a higher pH optimum, it was thought that the difference in pH levels may have accentuated the differences, especially for the bacterial amylase. Therefore, the three enzymes were tested by the liquefying method at pH values from 4 to 8. Little difference in activity was found between the values of 4.75 and 6.0. It is concluded that the difference in pH levels does not significantly contribute to the difference between the methods reported herein.

One of the objects of this work was to devise a satisfactory method for intercomparison of the three types of enzymes. It does not seem possible to intercompare malt and bacterial alpha-amylases by the two different methods. Since the liquefying method gives the highest value of the relative strengths, it is perhaps preferable to use this value as representing the maximum activity that can be obtained from a given weight of enzyme preparation. A more extended study of the dilution curve of fungal amylase was made and the results are given in Table X. It was found that between the limits of 15 to 50% decline in viscosity the relative strength of the fungal amylase compared with barley malt is reasonably constant. This can be used as

TABLE X

RELATIVE STRENGTH OF TAKADIASTASE B COMPARED TO MALT SYRUP A

Decline in viscosity		Relative strength
%		
15		25.4
20		28.0
25		28.4
30		28.4
35		28.4
40		27.7
45		26.0
50	Avg. = 26.9	23.3
55		20.0
60		17.0
65		15.0
70		13.1
75		11.8
80		10.8

a means of securing some agreement with the malt and bacterial amylases.

If the strength is measured in this range, the value will be a maximum. In general, it is desirable to measure enzyme strengths on a basis of initial rates as nearly as possible. The liquefying method is superior in this respect to the dextrinizing method since very little change of the starch takes place during the liquefying period.

It is not to be expected that the maximum enzyme values, as determined in the laboratory under a very specialized set of conditions, will necessarily agree with the relative values determined under plant or practical conditions. The best that we can hope to do in the laboratory is to devise a measure of the number of enzyme molecules that are present. How this number of enzyme molecules will work under a different set of conditions has to be determined experimentally.

Summary and Conclusions

A comparison of the relative liquefying and dextrinizing activities of alpha-amylases from three different sources, malt, bacteria, and mold, was made.

It was found that the relative strengths determined by the liquefying method were independent of the degree of liquefaction for the malt and bacterial amylases. They were not independent for the fungal amylases. However, the relative strength of the fungal amylase was found to be a maximum in the range 15 to 50% decline in viscosity, and measurements in this region are proposed as a measure of the maximum strength obtainable from a fungal amylase.

Liquefaction rate curves for equivalent alpha-dextrinizing amounts of the three types of amylase were not superimposable, which they should have been, if the two methods are equivalent. It is not strictly possible to intercompare the different amylases by the two methods, although on the basis of initial liquefying rates there is reasonable agreement for the malt and fungal amylases. If measurements are restricted to one type of amylase, they may, however, be intracompared by either the liquefying or alpha-dextrinizing method. Bacterial and fungal amylases may also be intracompared by a simple Wohlgemuth iodine method.

The alpha-amylases of malt, bacteria, and mold are a class of enzymes which differ quantitatively in their action on starch. (It is self-evident that pancreatic amylase is also a member of this class, but no experimental work was reported because of the considerable difference in optimum pH.)

Acknowledgment

The assistance of Mrs. Charlotte Gluck in making the dextrinizing determinations and of Mr. Leonard Wender in doing most of the liquefying work is greatly appreciated. The counsel of Dr. William R. Johnston was a source of constant encouragement.

Literature Cited

Blom, J., and Bak, A.
1938 Über die Bestimmung von Amylasen durch Verflüssigung von Stärkekleister. Z. physiol. Chem. 256: 197–207.
Dickson, A. D.
1943 A collaborative study of methods for the determination of amylase activity. Cereal Chem. 20: 31–35.
Hanes, C. S., and Cattle, M.
1938 Starch-iodine coloration as an index of differential degradation by the amylases. Proc. Roy. Soc. (London) B 125: 387–414.
Hollenbeck, C. M., and Blish, M. J.
1941 Parallelism between starch dextrinizing and liquefying activities of amylases. Cereal Chem. 18: 754–771.
Jozsa, S., and Johnston, W. R.
1935 Determination of alpha-amylase. Ind. Eng. Chem. (Anal. Ed.) 7: 143–146.
Kneen, E., Sandstedt, R. M., and Hollenbeck, C. M.
1943 The differential stability of the malt amylases separation of the alpha and beta components. Cereal Chem. 20: 399–423.
Landis, Q.
1943 Subcommittee report. Malt analysis committee. Read at Annual Convention, May, 1943.
1945 Methods for determination of alpha-amylase. I. Classification of enzyme assay methods, standardization of samples, and calibration of modified procedures. Cereal Chem. 22: 1–11.
Pigman, W. W.
1943 Occurrence of sucrose and inulin-hydrolyzing enzymes in commercial enzyme preparations. J. Research Nat. Bur. Standards 30: 159–175.
Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. 16: 712–723.
Wohlgemuth, J.
1908 Über eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments. Biochem. Z. 9: 1–9.

SUPERCENTRIFUGATES FROM DOUGH

J. C. Baker, H. K. Parker, and M. D. Mize

Wallace and Tiernan Laboratories, Newark, New Jersey

(Presented at the Annual Meeting, May 1944; received for publication January 30, 1945)

During the mixing of bread dough much of the water remains free to dissolve flour solubles, as suggested by Newton and Gortner (1922), and later determined by Skovholt and Bailey (1935) and Vail and Bailey (1940). This aqueous solution doubtless forms a liquid phase in the dough structure. The object of this paper is to describe a method of separating an aqueous solution from dough and the determination of its composition and properties.

The solubles in dough have heretofore been studied only in dilute solutions made by extracting flour, or leaching doughs dispersed in water. In recent studies on pentosans by Baker, Parker, and Mize (1943) and on sulfhydryl compounds of flour by Baker, Parker, and Mize (1944), flour solubles in a more concentrated form were obtained from centrifuged batters. The action of a high speed centrifuge on these batters suggested that some of the liquid phase of dough could be obtained in an unaltered and undiluted condition by centrifuging the dough in a higher speed machine. A Sharples centrifuge operated at 40,000 rpm using 200 g of dough in a closed bowl gave the desired results. The work here reported concerns the liquid which was drained from the surface of the supercentrifuged dough, the remainder of which remains firmly packed on the walls of the bowl.

Working (1934) previously supercentrifuged batters to determine absorption, but used less severe centrifugation since the dough remaining in the machine was presumed to retain the optimum amount of water for baking bread.[1]

The liquid obtained by supercentrifuging dough was analyzed and its properties determined. Variations among flours and the changes resulting from dough operations in breadmaking or from special ingredients added to dough or to flour were determined. The relation of the liquid phase of dough to breadmaking was further studied by using the supercentrifugate as the liquid for the manufacture of bread.

Methods and Materials

Preparation of Doughs. Doughs were made from a variety of flours in a closed McDuffy bowl with a Hobart C 10 mixer in the presence of carbon dioxide. A speed of 65 rpm was maintained for stated periods of time. An absorption of 73% was used for all flours corrected to 12% moisture. This amount of water was required to provide sufficient liquid from a single supercentrifugation so that all desired analytical data could be obtained. Many flours at normal absorption yield only small amounts of liquid. Also, this uniform absorption permits direct comparison of all data.

---

[1] The speed of the centrifuge at the time of operation was not stated by Working, but in a private communication Dr. C. O. Swanson advised that a speed of 10,000 rpm was used.

For all fermented doughs the following formula was used: 200 g of flour at 12% moisture basis, distilled water to 73% absorption, 4 g of salt, and 5 g of yeast. In nonfermented doughs the yeast was, of course, eliminated from the above formula.

Preparation and Analysis of Supercentrifugates. Immediately after mixing, 200 g of dough was supercentrifuged in the closed bowl of a Sharples supercentrifuge at a speed of 40,000 rpm for 20 minutes. The centrifuge was mounted in an insulated enclosure, the temperature of which was controlled so that the dough which left the mixer at a temperature of 29.4°C was held within ± 1°C during centrifugation. Full air pressure was applied intermittently to distribute the dough and obtain smooth operation, after which 30 pounds of air pressure was applied to operate the machine at full speed for 20 minutes.

During the operation part of the starch was forced to the bowl wall, gluten and starch formed an intermediate layer, and the free, very viscous liquor was crowded to the inner surface. Upon completion of the supercentrifugation the liquor was separated by gravity drainage for 2 minutes and weighed.

A gel test which, in part, was previously described by Baker, Parker, and Mize (1943) was performed on 3 ml of the supercentrifugate as follows: The liquor was forced from a pipette as a jet into 2 drops of 3% hydrogen peroxide contained in a 25 ml test tube. This procedure gave a uniform mixture. After an hour of contact, the rigidity of the gel was judged empirically, employing values from 0 to 8.

The liquor as originally separated could not be accurately sampled because of its high viscosity and the presence of small amounts of suspended material. Dilution with distilled water formed a precipitate. It was therefore diluted with 2 volumes of a 2.67% salt solution, this being the average concentration produced from the water and salt added to the dough. No precipitate was produced thereby. The traces of suspended matter were readily removed from the diluted solution by further centrifugation in covered cups. Weighed samples of about 5 g of the diluted solution were used to determine solids, pentosans, protein, and sodium chloride. All determinations were made in duplicate and results averaged. Solids were determined by evaporating a weighed sample of approximately 5 g to constant weight on a steam bath. Soluble pentosans were determined as outlined in *Cereal Laboratory Methods* (4th ed., 1941, p. 68) by distilling once. Preliminary tests indicated that redistillation did not appreciably lower the values. Soluble protein was determined by the Kjeldahl method as specified in *Cereal Laboratory Methods* (4th ed., 1941, p. 34). To determine the salt content, the dried soluble extract was ashed in the presence of 5 g of a mixture of 6 parts potassium carbonate, 4 parts sodium carbonate, and 3 parts potassium nitrate. This ash was then extracted with nitric acid and the chloride content determined, as described in *Cereal Laboratory Methods* (4th ed., 1941, p. 129). This result was corrected for the salt added in the dilution water. All results were calculated to percentage by weight of the original supercentrifugate.

The percentage of sugar was calculated from the difference between soluble solids and the sum of the soluble pentosan, soluble protein, and sodium chloride.

The viscosity of the diluted liquor recorded in seconds was determined in an Ostwald-Fenske pipette (Cannon and Fenske, 1936) and characterized by the number 300 with a water effluent time of 5 seconds. All measurements were made at 30°C.

The sulfhydryl determinations were carried out upon 0.5 ml of the diluted supercentrifugate as described by Baker, Parker, and Mize (1944) and calculated as ppm of glutathione in the original supercentrifugate.

The relation of sodium chloride to water was calculated from the total sodium chloride and water found in the original supercentrifugate. This calculation is made by the division of the percentage of sodium chloride by the sum of the percentage of sodium chloride and percentage of water and the multiplication of this quotient by 100. The percentage of water is determined by subtracting the percentage of soluble solids from 100%.

The resistance to puncture of hand-washed glutens and of glutens purified by two dispersions, both being in 2.67% sodium chloride solutions, was determined as described by Baker, Mize, and Parker (1943).

Results from Different Flours

The supercentrifugates from a series of 25 flours were prepared and analyzed. The results are reported in Table I showing the average, maximum, and minimum values obtained from these analyses. The analytical results obtained are not reported for each flour because of their voluminous nature. A series of five flours is reported to show some of the variations found in individual flours. It is not the in-

TABLE I

COMPOSITION OF SUPERCENTRIFUGATES SEPARATED AT ONCE FROM DOUGHS MIXED 1½ MINUTES
(Averages from 25 flours of 1943 and 1944)

Flour	Flour protein 12% moisture	Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	SH as G-SH	Force to puncture gluten	
												Crude	Pure
	%	g		sec	%	%	%	%	%	%	ppm	g	g
Average of 25 flours	12.2	23.8	6.5	30.8	13.1	6.61	1.55	2.61	2.33	2.62	180	10.2	28.2
Maximum	14.9	37.5	8.0	105.0	18.0	10.98	2.13	3.41	2.60	2.92	250	16.7	35.7
Minimum	9.8	8.8	0	9.0	10.1	4.10	1.09	1.99	2.12	2.43	130	4.2	15.1
REPRESENTATIVE FLOURS													
Thatcher Minot, 1944	12.9	13.1	8.0	64.0	13.4	6.40	1.87	2.85	2.28	2.57	220	14.0	32.1
Durum, 1943	14.9	26.1	7.0	14.0	18.0	10.98	1.49	3.41	2.12	2.52	190	5.1	15.1
Tenmarq, 1944	9.9	25.5	6.0	31.0	12.1	6.22	1.39	2.16	2.33	2.59	130	12.0	31.1
Chiefkan, 1943	12.8	14.3	6.0	38.0	13.4	7.02	1.62	2.50	2.26	2.54	170	9.4	26.9
Baart, 1943	9.8	33.7	8.0	13.0	12.5	6.30	1.39	2.46	2.35	2.61	210	4.8	17.2

tention of the authors to indicate that these supercentrifugates are necessarily typical for the varieties used.

The results found in these 25 patent flours of 9.8 to 14.9% protein content, milled experimentally or commercially, show very wide variations in the amounts of supercentrifugate obtained, the gelling property of this liquor, and its viscosity. In some cases, the total constituents of the liquor from different samples varied approximately twofold in amount. Soluble protein values varied somewhat less and carbohydrates somewhat more than the other constituents. Sodium chloride, which was an added ingredient in the dough, showed a variation which was greater than could be accounted for by the effect of the amounts of the other constituents. When the relationship of salt to water was calculated, a variation of 2.43 to 2.92% was found. If all the water in a dough were utilized in dissolving salt, the concentration would be 2.35%. If only the water added in making a dough from these flours of 12% moisture were utilized in dissolving salt, the concentration would be 2.67%. Hence, it would appear that in some of the doughs all of the added water plus a portion of the flour moisture is used in dissolving salt, whereas in other doughs less water than was added is utilized as a solvent. These variations can also be explained as the result of differences in amount of bound salt. The reasons for these wide differences in salt concentration are under further investigation.

The force required to puncture the gluten in the Baker apparatus is reported in all of this work on both crude and purified glutens to help characterize the flours. A variation of 4 to 1 in resistance to puncture was obtained on the crude hand-washed glutens. These, upon purification by two dispersions in the Waring Blendor, become at least twice as resistant with an approximate 2 to 1 variation.

Although the summary data would suggest there is a relationship among some of the properties and ingredients, such as viscosity and the yields of liquor and of pentosans, inspection of the individual results obtained, but not shown here, does not support this hypothesis. Inspection of the results from individual flours indicates a lack of correlation between any of the other values. The frequent absence of the gelling property previously reported on centrifugates from 2 to 1 batters by Baker, Parker, and Mize (1943) is not confirmed by this present work. Among the 25 flours inspected here, where the concentrated extracts separated from doughs were tested, only one yielded liquor that was not gelled at the end of one hour. This flour, though it gelled at once when tested, liquefied during the hour. These results indicate that an undiluted concentration of the pentosans is required to determine gelling tendencies and that this property is present in all refined flours.

Results from Dough Operations

The method of separating the liquid phase from dough by supercentrifuging afforded an opportunity of studying the effect of the operations of breadmaking upon its constituents and properties.

For this purpose, from each of the same 25 flours, four different doughs were prepared with 73% absorption and 2% salt, as listed in the left-hand double column of Table II. The first dough, labeled "minimum mix—at once," was mixed 1½ minutes, this being the minimum time required to wet completely the entire mass. This dough was then supercentrifuged at once. The second dough, labeled

TABLE II

PROPERTIES OF SUPERCENTRIFUGATES FROM 25 FLOURS OF 1943 AND 1944

Dough operation		Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	SH as G SH	Gluten puncture	
How mixed	When centrifuged											Crude	Pure
		g		sec	%	%	%	%	%	%	ppm	g	g
Minimum 1½ min.	At once	23.8	6.5	30.8	13.1	6.61	1.55	2.61	2.33	2.62	180	10.2	28.2
More than minimum	At once	22.4	6.3	32.5	13.4	6.87	1.59	2.62	2.32	2.62	177	10.0	27.5
More than minimum	After 3 hr.	28.1	6.0	29.0	15.0	8.45	1.68	2.58	2.20	2.63	179	10.2	26.4
More than minimum with yeast	After 3 hr.	30.7	5.7	30.0	10.5	4.15	1.65	2.34	2.36	2.61	167	12.0	28.8

"more than minimum—at once," was prepared by mixing as long as required for the optimum development of a dough from the flour at normal absorption. This procedure was used because of the extraordinary long mixing required at the 73% absorption by a few of the doughs which developed heat and also permitted considerable enzymatic action. The resulting doughs were thus all undermixed to varying degrees. The third yeastless dough was exactly like the second, with the same amount of mixing, but was allowed to stand 3 hours before centrifuging. The fourth dough was similar to the third except that it contained 2.5% yeast.

In comparing these results, the difference between the first and second doughs shows the effects of mixing; between the second and third doughs is shown the effects of autolysis in a yeastless dough upon standing for 3 hours. The fourth dough shows the effects of the yeast as distinguished from the autolysis in the third dough. A fifth comparison, showing the difference between the mixed dough centrifuged at once and the fermented 3-hour dough, gives the over-all inclusive effect during fermentation for 3 hours.

The dough operations do not alter appreciably the physical properties of the separated supercentrifugate except the quantity obtained. A large increase in the amount of the separated liquid phase of dough was found where yeastless doughs had stood 3 hours before centrifuging. A further increase was obtained where these doughs had been fermented. Tests, not reported here, show that most of the increase in liquor volume occurs in the first hour and is not altered by ordinary dough treatments. The action is somewhat similar to that observed in synaeresis and indicates by the unchanged composition that the dough materials pack more closely in the centrifuge after they have had a period of rest. The gel value and the viscosity are but little changed.

The changes in soluble solids are largely due to changes in carbohydrates shown in the succeeding column (Table II). The small increase in sugar occurring during mixing is doubtless due to the rapid attack of the diastatic enzymes upon broken starch granules. There was a further increase of 1.6% in the liquor during 3 hours. During fermentation a large portion of the total sugar in the liquor was consumed, averaging 4.3%.

Soluble pentosans show a tendency to increase in amount during mixing and standing, whereas yeast does not appreciably further affect them.

Soluble protein is substantially unaffected by all operations in breadmaking except fermentation, during which the yeast consumes some of the nitrogen materials in its metabolic processes. The apparent decrease in 3 hours is due to the effect of carbohydrate increase on the percentage.

The amounts of salt given as percentage of the dough liquid are not clearly significant because the concentration is affected by the amounts of other materials present in the dough. Hence, calculation has been made as entered in the succeeding column, showing the percentage of salt in the water of the supercentrifugate. This relationship of the averages remains substantially constant throughout all dough operations and thus indicates that the changes in dough properties encountered in breadmaking are not due to changes in the amounts of free and bound water in the dough. This has been shown also by Kuhlmann and Golossowa (1936). The liquor from individual flours may show considerable effect from dough operations, but these variations are peculiar only to each flour and do not disprove the generalization from the averages that the handling of dough in breadmaking produces its effects on dough properties by other changes than can be found in the liquid phase.

The sulfhydryl compounds are not much affected by dough operations excepting by fermentation where a very definite decrease in their average concentration occurs. A few flours, however, show no change. Thus, the conclusions reported by Hullett and Stern (1941) were usually, but not always, confirmed.

Crude gluten is strengthened by fermentation, but other dough operations show no effect. However, the method of preparing crude gluten is uncertain, so that more conclusive indications possibly are to be found in the purified glutens. These results indicate that there is a small drop in the resistance of gluten to puncture both with mixing and time. This loss is more than recovered during fermentation.

Aging of Flour

The aging of flour is known to produce very definite changes in its baking properties. The method of separating the liquid phase in dough offers an opportunity to study the effect of age on the liquid phase. From 1943 wheat, 16 flours, either mill produced or experimentally milled, were available for the study of age. The flours were analyzed and then stored for a 6-month period at 1°C in closed metal containers. Probably, under this condition of storage, oxygen was partially replaced by carbon dioxide from respiration, as reported by Working (1936). The results are thus not entirely analogous to those obtained in ordinary commercial storage. In order to study the effect of age, supercentrifugates from the four dough operations were separated from these flours when fresh and after 6 months' storage. Table III gives these results. The effects of 6 months' storage can

TABLE III

PROPERTIES OF SUPERCENTRIFUGATES FROM 16 FLOURS OF 1943

Dough operation		Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	-SH as G-SH	Gluten puncture	
How mixed	When centrifuged											Crude	Pure
		g		sec	%	%	%	%	%	%	ppm	g	g
AS RECEIVED													
Minimum 1½ min.	At once	20.1	5.0	27.1	13.5	7.02	1.51	2.56	2.41	2.71	178	9.4	26.3
More than minimum	At once	25.8	4.9	25.6	13.8	7.37	1.54	2.59	2.40	2.71	178	9.4	27.1
More than minimum	After 3 hr.	31.4	4.1	27.4	15.7	9.09	1.67	2.56	2.38	2.71	190	9.4	26.6
More than minimum with yeast	After 3 hr.	33.6	3.8	24.6	11.3	4.96	1.62	2.32	2.40	2.70	168	13.5	27.3
AFTER 6 MONTHS' STORAGE													
Minimum 1½ min.	At once	27.1	7.0	24.0	13.5	7.14	1.48	2.54	2.34	2.63	173	10.0	25.9
More than minimum	At once	25.3	7.2	27.0	13.7	7.38	1.48	2.50	2.34	2.64	179	9.2	25.4
More than minimum	After 3 hr.	32.3	5.0	24.0	15.3	8.79	1.62	2.53	2.36	2.64	192	9.3	25.3
More than minimum with yeast	After 3 hr.	34.0	5.6	22.0	11.0	4.93	1.50	2.23	2.34	2.64	172	13.4	26.5

best be observed by comparing the values for each property or ingredient from the respective operations. The results indicate that the physical properties of the dough supercentrifugate were not greatly changed by flour storage with the exception of gel value, which became definitely stronger. The amount of carbohydrates found in the liquor from the aged flour was slightly less where the dough was permitted to stand 3 hours before separation, thus indicating that some diastase had disappeared from the flour during storage. There were also small decreases in the amounts of soluble pentosan and soluble protein. These decreases are particularly noticeable in the fermented samples. Possibly the alcohol from fermentation has a greater effect upon the solubility of pentosans of flours stored 6 months. Soluble protein is slightly more available for yeast metabolism in the aged sample. The most striking change in these doughs is found in the relation of salt to water; the aged samples furnish a more dilute salt solution, which indicates that there is either less bound water in these doughs or more bound salt. That the latter is more likely true is indicated by comparing the analyses of the fresh and aged samples mixed 1½ minutes and centrifuged at once. The constituents in these two sets are substantially identical in amounts, thus suggesting that no change in the water binding capacity has occurred over the 6-month storage period. Thus, the lowered salt concentration would appear to be due to a decrease in the amount of salt in solution, indicating that salt has been taken into or combined with some of the other dough ingredients.

The sulfhydryl determinations show no change over the 6 month period. This surprising result is probably due to the samples being stored at 1°C in closed metal containers.

The resistance to puncture of the purified glutens in all operations shows a loss of about 1 g, indicating small decreases in gluten strength over the 6-month period.

Effects of Nitrogen Trichloride and Potassium Bromate

The action of nitrogen trichloride and potassium bromate was studied on a series of 15 flours produced from 1943 and 1944 wheats. The amounts of nitrogen trichloride used on these samples varied from 0.5 to 1.5 g per cwt, depending upon their effects in the baking tests. The amounts of bromate used varied from 10 to 30 ppm for the same reason. The analysis of liquor separated from fermented doughs only is reported. Tests were made on doughs from untreated flour, from flours treated with nitrogen trichloride, and also on similar sets of doughs to which bromate was added. These results are given in Table IV. The only appreciable change in the physical properties of the various supercentrifugates is a slight increase in viscosity in the nitrogen trichloride treated samples. This is hardly large enough to be significant. The amount of carbohydrates in solution clearly indicates that neither reagent has affected diastatic action in these doughs. This result is somewhat surprising and gives no explanation

TABLE IV

COMPOSITION OF SUPERCENTRIFUGATE FROM 3-HOUR FERMENTED DOUGHS TREATED WITH POTASSIUM BROMATE OR MADE FROM NITROGEN TRICHLORIDE TREATED FLOURS

(Average of 15 flours of 1943 and 1944)

Treatment	Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	SH as $Cl\cdot SH$	Gluten puncture	
											Crude	Pure
	g		sec	%	%	%	%	%	%	ppm	g	g
AVERAGE OF 15 FLOURS OF 1944												
Untreated	30.7	5.9	31.6	10.1	3.85	1.66	2.30	2.29	2.54	166	13.6	30.8
+Nitrogen trichloride	31.9	5.9	32.7	10.1	3.91	1.62	2.29	2.28	2.54	184	13.7	30.5
+Potassium bromate	31.2	6.1	30.6	10.1	3.88	1.64	2.29	2.29	2.55	159	12.6	27.7
+Nitrogen trichloride and potassium bromate	30.4	6.2	32.4	10.1	3.90	1.60	2.24	2.27	2.53	151	13.3	27.4

for the changes in crust color sometimes observed when baking nitrogen trichloride treated flours. No appreciable action was found on soluble pentosans or proteins or the distribution of water and salt in the dough system. The sulfhydryl compounds were definitely lowered by the oxidizing agents, the nitrogen trichloride treatments giving the greater effect on these fermented doughs.

A more complete study of the action of nitrogen trichloride on sulfhydryl compounds in flour is shown in Table V. Here, the amounts of sulfhydryl, reported as ppm of glutathione in the liquid phase, are shown for the dough operations used in Tables II and III. It is to be noted that the moderate treatments with nitrogen trichloride have produced no change in the sulfhydryl content of these 15 flours until they were fermented. The action of the yeast upon the untreated flours produced a decrease of approximately 14 ppm, whereas on the treated flours a decrease of 33 ppm was obtained. These results indicate that the action of nitrogen trichloride upon sulfhydryl compounds is to alter them in some manner so that yeast has a greater action upon them during fermentation. Baker, Parker, and Mize (1944) have previously shown that very heavy treatment with nitrogen trichloride lowers the soluble sulfhydryl content of unfermented dough.

TABLE V

SULFHYDRYL COMPOUNDS IN SUPERCENTRIFUGATES FROM DOUGHS MADE FROM NITROGEN TRICHLORIDE TREATED FLOURS SEPARATED AT DIFFERENT STAGES OF DOUGH HANDLING

(Average of 15 flours of 1943 and 1944)

Dough operation		Untreated	NCl_3 treated
How mixed	When centrifuged	-SH as G-SH	-SH as G-SH
		ppm	*ppm*
Minimum	At once	177	179
More than minimum	At once	178	175
More than minimum	After 3 hr.	180	187
More than minimum with yeast	After 3 hr.	166	154

The action of bromate upon the strength of gluten during fermentation is shown in Table IV. A loss occurred in resistance to puncture of about 3 g, amounting to 10%. This decrease confirms results previously published by Baker, Mize, and Parker (1943), which showed that bromate lowered the resistance of gluten to puncture.

Effect of Different Crop Years

The data presented show samples separately analyzed from the 1943 and 1944 crops. The top row of figures in Table IV labeled "untreated," and the fourth row in Table III labeled "more than minimum with yeast after 3 hr.," both on new flours, are directly comparable. The liquor of the 1943 crop, as compared to the 1944, had more diastase, formed a stronger gel, had a lower viscosity, a higher concentration of salt in the water of the liquor, and yielded a weaker gluten on purification. These results do not necessarily mean that there was this difference generally in the two crop years, because there are not enough samples selected to establish this as a fact. They are compared here, however, as being suggestive of differences which may be encountered.

Baking Results with Supercentrifugate

Some evidence as to the action of flour solubles on dough properties can be obtained by the use of dough liquor as such to furnish the required water in breadmaking. The concentration of flour solubles in the dough is thus approximately doubled. These experiments were made with a northwestern spring wheat patent flour of 11.9% protein and 0.39% ash, using the straight dough process. It so happens that enough salt and sugar are present in the solution from this flour to supply the amounts needed for breadmaking. Hence, corresponding amounts of salt and sugar were added only to the doughs made with water. The excess materials present in the doughs made with supercentrifugate are soluble pentosans, proteins, sulfhydryl compounds, and enzymes. The total water added to the doughs was held constant regardless of whether water or supercentrifugate was used. Equal weights of dough were baked. The effects upon the properties of dough and bread are marked and readily observed and are summarized from tests on many other flours. Doughs become softer and more yielding though not overmixed in character. "Buckiness" disappears and in some cases the dough may even possess the character of excessive flow. Immediately after such doughs are mixed, excessive stickiness is observed, but this disappears during fermentation. They mold and shape more easily. During proof the doughs rise more rapidly but with less boldness. The excessive flow and surface stickiness produced by the supercentrifugate in the freshly mixed dough suggested that the absorption should have been decreased. The dryness of the dough when molded and the character of the finished bread indicated that even more water could have been used without damaging the bread had it been possible to handle the doughs at time of mixing.

When baked, the bread made with supercentrifugate has larger volume with greater oven spring and a richer colored crust, as shown in Figure 1.

The two loaves on the left were made with distilled water without and with bromate. Those on the right are corresponding loaves, in which supercentrifugate supplied the same amount of water. It is to be noted that the latter loaves are larger with finer grain in both instances and gave a positive response to 20 ppm bromate. The control loaves, on the contrary, were distinctly damaged by this comparatively large bromate treatment. The liquor ingredients have modified or protected the dough in such a manner that bromate has produced an improvement without injurious effect. This protection is similar to the action of milk, as reported by Larmour, Working, and Ofelt (1939) and later shown by Baker, Mize, and Parker (1943) specifically to protect gluten from damage. The improving effects obtained from supercentrifugate in these experiments are due to other ingredients than enzymes, for even better results have been obtained where the liquor has been heated to 80°C before use.

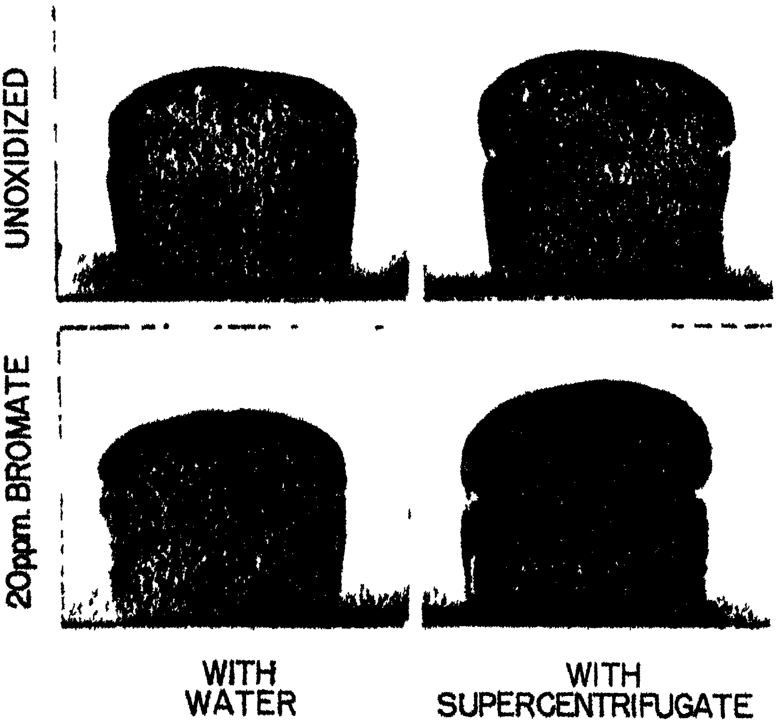


Fig. 1. Effect of supercentrifugate in breadmaking.

Discussion

In dough the liquor phase must be the medium through which all reactions occur during manipulation and fermentation. Gluten and starch are permeated by and suspended in the liquid medium. The liquid phase becomes the medium through which changes occur, and dough properties are altered because only through it can additions or withdrawals of ingredients from gluten or starch take place. Thus, the analysis and examination of dough liquor should disclose or indicate the reactions that are responsible for dough changes.

Doubtless there remain other factors in dough liquor than those determined, such as enzymes, metabolic products of yeast, and protein and starch degradation products, which play an important role. The data given, however, largely indicate the extent of these changes and their influence can be interpreted.

The changes in dough liquor properties, which the average values presented here indicate, do not occur in all cases so that one cannot necessarily infer the changes which are going to occur in a given dough. The changes from fermentation are such as one would expect. Sugars, soluble protein, and sulfhydryl compounds disappear, alcohol and carbon dioxide are produced. A physical change occurs in dough with time that permits more liquid to separate. This plus the gelling property of the liquid and changes in gluten strength induced by the chemical changes may largely account for variations found in dough during fermentation.

One observation from this work indicates that proteolysis plays a very small part, if any, in dough reactions where sound, refined flours are used. No significant changes are found in soluble protein or gluten strength of doughs which have stood 3 hours without yeast. Fermentation, however, shows a relatively large effect upon these protein components, thus indicating that the changes found in fermented doughs are due to causes other than proteolytic enzymes from flour. The softening of yeastless doughs, which has so often been taken as a measure of proteolysis, is apparently a physical change in dough properties resulting from rearrangement of components. In the softened doughs the solids pack tighter in the centrifuge. All that is required to explain the softening change is the relaxation of the gluten micelle from the strained condition produced by mixing.

All sulfhydryl compounds exist naturally in an unreactive form in native protein and are measurable only after complete denaturation with guanidine hydrochloride (Neurath, Greenstein, Putnam, and Erickson, 1944). Unfortunately, the sensitivity of the method is too low to yield accurate results with such very low concentrations. Undoubtedly changes in sulfur have occurred which it was impossible to detect. Some flours showed no detectable change in any dough operation. This surprising result may, in part, be due to the weakness of the method but still shows clearly that some flours are very resistant to changes in their sulfhydryls. In six fermenting doughs no loss of sulfhydryl was detected. This result indicates that the conclusions of Hullett and Stern (1941) may not be universally true.

The method of separating a liquor from dough in the supercentrifuge offers an opportunity of studying many other variables in flours and doughs not reported here. The effects of temperature changes and absorption or of other dough ingredients, such as sugar, milk, enzymes, etc., could well be studied by this method. Other treatments of flour and methods of handling dough might yield interesting results. The action of heat on dough could be studied up to the point of starch swelling.

Summary

A procedure has been described for the separation of a liquor from doughs of 73% absorption by means of the Sharples supercentrifuge operated at 40,000 rpm.

Supercentrifugates so obtained have been prepared from doughs made with 25 different flours subjected to four distinct dough operations. These liquors have been analyzed for soluble solids, pentosans, proteins, (–SH), and salt content, and have been tested for yield, viscosity, and gel strength. The analyses and tests show the comparative effects of mixing, 3-hour standing, and 3-hour fermentation upon all flours studied.

Little correlation was found between amounts of supercentrifugate ingredients themselves, between the amounts of liquor obtained and the composition of the liquor, or between properties of the dough liquor and concentration of its ingredients. The action of nitrogen trichloride and bromate has been shown to be largely due to either changes in physical properties of gluten or in soluble sulfhydryl compounds. Flour storage results in an increase in salt binding capacity. Dough operations before baking, as well as flour or dough oxidizing treatments, do not alter the water or salt binding capacity of flour substances.

Acknowledgment

The authors are indebted to Dr. E. G. Bayfield and Dr. J. H. Parker for the experimentally milled Kansas Type wheat flours, and also to the Northwest Crop Improvement Association for the samples of the experimentally milled northwestern spring wheat flours, which were used in this study.

Literature Cited

Baker, J. C., Mize, M. D., and Parker, H. K.
1943 Measurement and significance of gluten quality. Cereal Chem. 20: 506–516.
— —, Parker, H. K., and Mize, M. D.
1943 The pentosans of wheat flour. Cereal Chem. 20: 267–280.
1944 The action of oxidizing agents on sulfhydryl compounds in dough. Cereal Chem. 21: 97–107.

Cannon, M. R., and Fenske, M. R.
1936 Equipment for accurately measuring viscosity in fundamental units. Oil and Gas J. 34 (47): 45–49.
Hullett, E. W., and Stern, R.
1941 Biological elimination of glutathione from wheat germ and flours used in bread making. Cereal Chem. 18: 561-572.
Kuhlmann, A. G., and Golossowa, O. N.
1936 Bound water in bread making. Cereal Chem. 13: 202 217.
Larmour, R. K., Working, E. B., and Ofelt, C W.
1939 Quality tests on hard red winter wheats. Cereal Chem. 16: 733 752.
Neurath, H., Greenstein, J. P., Putnam, F. W., and Erickson, J. O.
1944 The chemistry of protein denaturation. Chem. Rev. 34: 157 267.
Newton, R., and Gortner, R. A.
1922 A method for estimating hydrophilic colloid content of expressed plant tissue fluids. Botan. Gaz. 74: 442-446.
Skovholt, O., and Bailey, C. H.
1935 Free and bound water in bread doughs. Cereal Chem. 12: 321-355.
Vail, G. E., and Bailey, C. H.
1940 The state of water in colloidal gels: Free and bound water in bread doughs. Cereal Chem. 17: 397–417.
Working, E. B.
1934 The determination of the water absorbing capacity of flour by means of the super centrifuge. Cereal Chem. 11: 567 568.
1936 Measurement of respiration in flour. Cereal Chem. 13: 234 236.

THE DISTRIBUTION OF WATER IN DOUGH

J. C. BAKER, H. K. PARKER, and M. D. MIZE

Wallace and Tiernan Laboratories, Newark, New Jersey

(Received for publication January 19, 1945)

Bread dough is produced by mixing together a solid and a liquid. When the two are combined to make dough the resulting mass has both solid and liquid properties. It is elastic, resists distortion, but will flow slowly with time. When enough water is added to a dough it becomes a fluid.

The theory usually accepted about the distribution of water in dough is that a large portion of it is free while the remaining portion combines with the flour constituents to form hydrates, or is bound by polar groups or otherwise reacted in such a manner that it is no longer available as a solvent. This theory of bound water has developed from the hypothesis advanced by Newton and Gortner (1922) and is in accord with Gortner's (1938) later views in respect to the state of water in biological fluids and in solutions of hydrophilic colloids. Alsberg (1927) quoted the earlier work of Rodewald (1896) and Boutroux (1897) who calculated that 82% of the water in dough is bound. Swanson (1943) estimated by a method of calculation similar to that of Boutroux that two-thirds of the water is bound. Kuhlmann and Golossowa (1936) found that the solids in flour bind 44.4% of their weight of water, as estimated by a refractometric method based on that of Dumanski (1933). The freezing point method of Newton and Gortner (1922) for the determination of bound water was adopted by Skovholt and Bailey (1935) and Vail and Bailey (1940) for dough measurements. These workers found that flour substance bound respectively 45.4% to 42.1% and 28.6%. Swanson (1943a) states that since there is no sharp line of demarcation between free and bound water, variations in the method of measurement may be a considerable factor in the divergent values reported.

Baker, Parker, and Mize (1945) have described a liquor separated from doughs by means of the Sharples supercentrifuge. The concentration of salt in this dough liquor should be a measure of the amount of water available in the dough for dissolving purposes and hence of the bound and free water content of the dough under actual dough conditions. These authors found the concentration of salt in supercentrifugates when calculated in relation to the water present varied from 2.43% to 2.92% in different doughs, thus indicating a possible wide variation in the distribution of water in doughs.

Experimental

In order to study the distribution of water in a dough, a commercial flour was selected which gave dough liquor of approximately average salt to water ratio. This flour was a hard winter patent containing 12% moisture, 11.8% protein, and 0.40% ash, as found. It was made into dough by adding 73% of its weight in water and 2% of salt. The composition of the dough was as follows:

48.6	g of water
37.5	g of starch
6.1	g of gluten
6.66	g of flour solubles
1.14	g of sodium chloride
100.00	g of dough

This dough was supercentrifuged in the manner described by Baker, Parker, and Mize (1945) for varying periods of time to determine whether the liquor forced from the dough was of uniform composition.

In Table I are listed the amounts and analyses of the supercentrifugate obtained from five separate centrifugings of the dough at 10, 20, 40, and 60 minutes and also that amount obtained in 60 minutes plus all the liquid that could be removed from the surface of the dough by a rubber policeman after drainage had been completed. Supercentrifuging for more than an hour produced no further appreciable separation of liquid. It is to be noted that the separation of liquor from the dough is substantially completed in 40 minutes and that the composition of the free flowing liquor as regards the amount

TABLE I

CHARACTER OF SUPERCENTRIFUGATE SEPARATED AFTER VARIED LENGTHS OF CENTRIFUGING

Supercentrifugate period	Liquor yield	Water in liquor[1]	Concentration of NaCl in liquor	Concentration of NaCl in water in liquor
	g	%	%	%
10 minutes	24.6	87.6	2.34	2.60
20 minutes	30.8	87.6	2.35	2.62
40 minutes	35.9	87.7	2.38	2.61
60 minutes	36.7	87.1	2.34	2.61
60 minutes plus very viscous liquid	39.9	86.7	2.34	2.63

[1] The water in the liquor was determined by evaporation on a steam bath to constant weight.

of water was constant at all periods of centrifuging. When the viscous material was scraped from the surface of the dough an increase in solubles was obtained, which was largely pentosans, the amounts of which are not reported here. The total amount of liquid removed in the experiment represented approximately one-half the water added in making the dough.

It is to be noted that irrespective of the time of centrifuging or the scraping, the relationship of salt to water was substantially constant in the successive portions of liquor, thus suggesting that the liquid remaining in the dough was of the same composition.

The amount of water used in making this dough, i.e., 73 g plus the 2 g of salt added, would make a solution containing 2.67% of salt. Liquor separated from the dough contained only an average of 2.62% of salt, thus suggesting that there is more free water in dough than was added in making the dough and indicating that either some of the flour moisture is present as free water or some of the salt is combined with dough ingredients and removed from the liquid system.

Action of Starch and Gluten. In order to find how much water the insoluble ingredients of flour may bind, experiments were conducted on starch and gluten under conditions simulating those in dough. Starch was washed, purified with distilled water, vacuum dried, and then studied for its water-binding characteristics by exposing to the vapor of a 2.62% sodium chloride solution and then determining the total amount of moisture in the starch after equilibrium was reached. Under these conditions, 100 g of starch contained 32.6 g of water at 85°F (29.4°C). In a similar experiment in which the starch was exposed over the supercentrifugate from the dough, it contained 30.1 g of water.

Similar experiments were conducted with gluten purified by dispersing it four times in 0.15% sodium chloride solution, as described by Baker, Mize, and Parker (1943). This purified gluten was dried in vacuum at 45°C and ground to 40 mesh. When suspended in the vapor of 2.62% salt solution, 100 g of gluten took up 40.9 g of water and when suspended over supercentrifugate from the above-described dough, the gluten absorbed 39.3 g of water. These results are summarized in Table II.

TABLE II

WATER BINDING ACTION OF STARCH AND GLUTEN

WATER BOUND BY 100 G OF DRY STARCH AT 85°F (29.4°C)	Water
From Vapor	
Over 2.62% sodium chloride solution.	32.6 g
Over dough liquor	30.1 g
WATER BOUND BY 100 G OF DRY GLUTEN AT 85°F (29.4°C)	
From Vapor	
Gluten over 2.62% sodium chloride solution	40.9 g
Gluten over dough liquor	39.3 g
WATER BOUND IN 100 G OF DOUGH AT 85°F (29.4°C) (CALCULATED)	
37.5 g of starch binds.	11.3 g
6.1 g of gluten binds.	2.4 g
Total bound water in dough	13.7 g

The amount of water bound in 100 g of this dough at 85°F (29.4°C) by starch and gluten can be calculated from the results obtained by suspending these ingredients in the vapor of dough liquor. This indicates that starch binds 11.3 g of water and gluten binds 2.4 g, giving a total of 13.7 g of water bound in the insoluble ingredients of dough. It would appear reasonable that the amount of water which these ingredients absorb when exposed to the vapors of dough liquor is the same as they take up in the dough itself, where they are immersed in a similar liquid, because the vapor pressure conditions are identical whether in the vapor or in the liquid and the equilibrium should be the same in both cases. It is possible that the small amount of salt which it was necessary to leave in the gluten for its preparation may cause it to absorb a little more moisture than would be taken up under actual dough conditions where no such excess of salt would be present.

If the indicated 13.7 g of bound water be calculated to the basis used by Skovholt and Bailey (1935), 100 g of dry flour binds 27.3 g of water. Vail and Bailey (1940), when working with salt-free doughs at freezing temperatures, determined that 100 g of dry flour binds 28.6 g of water. These similar results suggest that bound water in dough is not greatly altered by salt and temperature.

Effect of Salt. The effect of salt on the amount and composition of the supercentrifugate obtainable from dough was tested by making doughs with their salt content varied from no salt up to 4% and supercentrifuging for 20 minutes. The amount of liquid, viscosity, total solids, and salt content was determined. The results are shown in Table III.

TABLE III

SUPERCENTRIFUGATE FROM 200 G OF DOUGH WITH SALT CONTENT VARIED, CENTRIFUGED 20 MINUTES[1]

NaCl used per 100 g flour	Yield	Viscosity	Soluble solids	Sodium chloride	Salt-free solubles[2]
	g	sec	g/100 g	g/100 g	g/100 g
None	17.7	43	10.4	0.09	10.3
0.125 g	23.4	31	10.3	0.17	10.1
0.25 g	24.6	31	10.8	0.35	10.4
0.50 g	28.5	25	10.7	0.64	10.2
1.00 g	31.2	23	11.4	1.30	10.2
2.00 g	30.7	25	12.5	2.61	10.2
4.00 g	27.1	27	14.3	4.63	10.2

[1] Methods of analysis used are described by Baker, Parker, and Mize (1945).

[2] Calculated by formula $\frac{\text{solubles} - \text{salt}}{100 - \text{salt}}$ = salt-free solubles in 100 g of salt free liquor.

The concentration of solubles in the liquor was calculated to its concentration in the water of the liquor, as shown in the last column. The uniform values obtained indicate that varying amounts of salt in dough have no effect upon the total amount of flour substances dissolved in the liquid phase. This result probably indicates why bound water determined in the presence of salt checks so closely with that of Vail and Bailey (1940), where no salt was used.

We have not been able to determine the effect of low temperature on the composition of supercentrifugate because of difficulty in holding lower temperatures in the Sharples machine. Neither was it possible to study absorptions at 60% as used by Vail and Bailey (1940), since little liquor separates from such dough. The similar results obtained by these different methods further suggest that the amount of water bound in dough is not greatly influenced by excess added water. The results of Baker, Parker, and Mize (1945) show that salt concentrations in the water of dough supercentrifugates vary from 2.43% to 2.92%. Hence, the agreement between our results and those of Vail and Bailey may be accidental and no definite conclusions can be drawn from the similarity.

Volume Relationships of Dough Phases. The gravimetric data on the bound water of dough do not clearly show its relation to the properties of dough. The relative volumes of the various dough phases should give a clearer picture of their relative action to each other in breadmaking. The volumes can be calculated from their amounts and densities. The same dough was used, the composition of which is given in the first part of this paper. The results of these calculations are shown in Table IV.

TABLE IV

CALCULATION OF PHASE VOLUMES IN 100 G DOUGH OF 73% ABSORPTION

Dough substances	Weight	Density	Calculated volume	Calculated phase volume	Calculated phase volume
	g		*ml*	*ml*	*%*
Dough 73% absorption	100.0	1.235	81.0	81.0	100.0
Starch dry	37.5	1.530	24.5		
Water bound in starch	11.3	1.000	11.3		
			35.8		
Hydrated starch				35.8	44.2
Gluten dry	6.1	1.300	4.7		
Water bound in gluten	2.4	1.000	2.4		
			7.1		
Hydrated gluten				7.1	8.8
Total hydrated insolubles	(35.8 + 7.1) =			42.9	53.0
Total liquid	(81.0 − 42.9) =			38.1	47.0
Liquid and gluten	(38.1 + 7.1) =			45.2	55.8

The last column gives the space occupied by the different phases in percent of total dough volume. Hydrated starch occupies 44.2%, hydrated gluten 8.8%, and the liquid fraction 47%. The combined volumes of the hydrated gluten and the liquid which together surround the starch amount to 55.8%.

Tightly packed wheat starch from this flour was determined to have a density of approximately 1.13, as packed. Since the density of starch is 1.53, the voids in this packed wheat starch are calculated to be 26% of its volume, or 11.5% of the dough volume. If the granules of starch were of one uniform size, the voids would have been 50% of its volume. The lower figure of 26% is obtained because starch contains a large proportion of very small granules which pack in between the larger granules and occupy the voids (Stamberg, 1939). Thus, there is required only about one-fifth of the gluten dough liquor mixture to fill this minimum of voids. Such an assembly would be substantially rigid because the starch granules would contact each other. The gluten and liquid mixture in dough, by its volume, holds the starch granules apart and the mixture becomes fluid. The starch granules are the solid bodies around which the gluten liquor mixture must move in dough as it responds to applied stresses. However, many of the smaller starch granules are tightly bound to gluten and must move with it in dough, thus producing an additional increase in resistance to distortion by dough.

When less water is used in making a dough, the concentration of the soluble solids in the liquor becomes greater and its viscosity increases. The gluten particles are brought closer together and the starch granules come nearer to each other. A marked increase in dough resistance to flow results. This crowding together of the gluten forces it into more contacts and thus results in quicker gluten reaction during mixing of doughs of less absorption. This is shown in the quicker rise of a mixogram curve, where less water is used.

Dough Liquor and Gluten Mixture. Calculations of Table IV show that 55.8% of the volume of this 73% absorption dough consists of gluten and liquid. The properties of such a mixture depend largely upon the amount and character of its gluten. The dough liquor plays a role in modifying these properties. This can be observed when gluten is washed from a dough by using starch-free dough liquor as the washing fluid. A great deal of care is required under this condition to hold the gluten together. A large soft mass of gluten impregnated with dough liquor is thus obtained which may possibly have characteristics similar to the mixture as it exists in dough. It is elastic but flows with astonishing ease under strain. It cannot be held in one piece when worked and squeezed as ordinary gluten but must be handled very gently. When centrifuged, liquor is forced out and the mass becomes firmer. If transferred to water its character is quickly changed to that of ordinary gluten as the solubles are removed. This is an irreversible process since ordinary gluten does not become softer upon working in dough liquor.

The strength of gluten increases when centrifuged or washed. Both processes enable gluten contacts and bonds to be established more readily. The maximum strength of gluten should thus be obtained when no free liquid or dough solubles are present. This condition is produced by exposing dry purified gluten to water vapor as already described. Finely ground gluten when it has hydrated to a state of equilibrium becomes cohesive, soft, plastic, and very strong, but has no quality of wetness. Such gluten possesses maximum strength and resistance to flow. It has no capillaries to hold a liquid phase and no pentosans or other solubles to lubricate or interfere with its cohesions.

Discussion

In view of the large amount of liquid present, it may be informative to consider bread dough as an aqueous medium which has its properties modified by the presence of flour. Flour batters are liquids. The addition of more flour converts them to dough. The fluid property of the batter remains in the dough. About three-fourths of the total water may remain as a viscous, liquid solution of the solubles. Starch binds a portion of the water, occupies about 44% of the gas-free dough volume, and constitutes the solid portion of the dough. Hydrated gluten, though largely fluid in its action, also has elastic properties from its cohesions and holds the fluid mass together. Dough thus contains a highly viscous liquid rendered slightly elastic by the bonds between the gluten micelle dispersed in the liquid. These properties are modified by an approximately equal volume of suspended starch which adds puttylike properties to dough.

Summary

When supercentrifuged for increasing periods of time, bread dough containing salt, but no yeast, yielded increasing amounts of liquid containing identical percentages of salt. This offered an opportunity for determining the amounts of free and bound water in doughs by analyzing the separated liquid for its salt and water content. With some flours the result indicated less bound water present in dough than there was moisture in the flour. This appeared improbable and suggested the method did not yield the correct result.

Therefore, the water-binding capacities of starch and gluten were separately determined by exposing purified salt-free samples to the vapor from dough supercentrifugate. When equilibrium was reached, the amounts of water absorbed were calculated. This indicated that the dry flour in dough binds 27.3% of its weight of water. This result is nearly the same as that found by Vail and Bailey (1940), who worked with salt-free doughs at 0°C.

The effect of salt on the distribution of water in dough was tested by preparing supercentrifugates from doughs of varying salt content from 0% to 4%. The addition of salt did not alter the concentration of flour solubles in the water of the separated solution, thus showing that salt in these concentrations does not alter the amount of free water in dough and hence also has no effect on the amount of bound water. This indicates that both Vail and Bailey's freezing point experiments and those of the authors with salt-free materials suspended in vapor give approximately correct results for the percentage of bound water in doughs containing salt. The volumes occupied in dough by the solution containing solubles and by the hydrated insolubles, gluten, and starch were calculated from their known amounts and densities. In this dough of 73% absorption, 47.0% of its volume was aqueous solution, 44.2% hydrated starch, and 8.8% hydrated gluten. That hydrated gluten and the aqueous solution are closely associated and move together in dough around the starch was shown by removing the starch from dough by a gluten washing procedure using starch-free supercentrifugate as the washing liquid. The final, tender separated gluten was impregnated with the dough liquid in amounts similar to those in dough. It would appear that such a mixture, which together comprises 55.8% of the dough volume, surrounds the starch and contributes fluid and elastic qualities to dough while the starch adds puttylike properties to the dough.

Literature Cited

Alsberg, C. L.
1927 Starch in flour. Cereal Chem. 4: 485-492.
Baker, J. C., Mize, M. D., and Parker, H. K.
1943 Measurement and significance of gluten quality. Cereal Chem. 20: 506-516.
——, Parker, H. K., and Mize, M. D.
1946 Supercentrifugates from dough. Cereal Chem. 23: 16-30.
Boutroux
1897 Cited by Maurizio, A., Die Nahrungsmittel aus Getreide. 2te Aufl. Vol. I, p. 349. Paul Parey, Berlin, 1924.
Dumanski, A.
1933 Die Bestimmung der Menge des gebundenen Wassers in dispersen Systemen I. Methode der Refraktometrie und Polarimetrie. Kolloid-Z. 65: 178-184.
Gortner, R. A.
1938 Outlines of Biochemistry. John Wiley and Sons, p. 278.
Kuhlmann, A. G., and Golossowa, O. N.
1936 Bound water in bread making. Cereal Chem. 13: 202 217.
Newton, R., and Gortner, R. A.
1922 A method for estimating hydrophilic colloid content of expressed plant tissue fluids. Botan. Gaz. 74: 442-446.
Rodewald, H.
1896 Untersuchungen über die Quellung der Starke. p. 70. Lipsius und Tischer, Kiel und Leipzig.
Skovholt, O., and Bailey, C. H.
1935 Free and bound water in bread doughs. Cereal Chem. 12: 321-355.
Stamberg, O. E.
1939 Starch as a factor in dough formation. Cereal Chem. 16: 769-780.
Swanson, C. O.
1943 Physical properties of dough. p. 111. Burgess Publishing Co., Minneapolis, Minn.
1943a Physical properties of dough. p. 94. Burgess Publishing Co., Minneapolis, Minn.
Vail, G. E., and Bailey, C. H.
1940 The state of water in colloidal gels: Free and bound water in bread doughs. Cereal Chem. 17: 397-417.

GAS OCCLUSION DURING DOUGH MIXING

J. C. Baker and M. D. Mize

Wallace and Tiernan Laboratories, Newark, New Jersey

(Presented in part at the 1943 Annual Meeting, received for publication February 2, 1945)

When studying the mixing of doughs in vacuum and in the presence of various gases, Baker and Mize (1937) observed that gases are progressively occluded in doughs during mixing. They measured the amounts of gas dispersed in a dough by comparing its density to that of the same dough mixed in vacuum. Doughs mixed in vacuum contained no voids or occluded gas and had a density of 1.24, whereas those mixed in gas had densities as low as 1.02 when given excessive mixing. Thus the overmixed dough contained gas equal to approximately 20% of the volume of the dough substance. At that time it was assumed that the stickiness and softness that often developed during excessive mixing might be explained by large amounts of occluded[1] gas. Later these same authors (1941) studied the origin of gas cells in bread dough and concluded that all cells found in bread originate from the operations which apply work to the dough, such as mixing, punching, rounding, and molding. It was shown that the yeast organism does not originate gas cells and that cells which are originated by work applied to dough may not be retained during subsequent operations unless the dough is in proper condition to avoid their loss.

The present study was prompted by the development of a simplified method for determining density whereby the occlusion of gases in dough could be rapidly followed throughout the course of the dough mixing period. The method was used to study the relationship between dough mobility, gas occlusion, and baking quality.

Method of Determining Density

The density of the dough taken directly from the mixer was determined by dropping small pieces into calcium chloride solutions of known density at 85°F (29.4°C) and observing whether the sample sinks or floats. Dough density can be readily determined by having a series of such solutions covering the range of densities encountered in dough mixing. The amounts of anhydrous calcium chloride required to prepare the solutions can be found in many handbooks. Calcium chloride solutions with densities varying between 1.24 and 1.00 cover the densities of doughs encountered in these studies, including those which were too badly overmixed for handling, as well as those which were mixed in vacuum.

---

[1] In earlier papers the words "emulsed," "emulsification," etc., were used to describe the incorporation of gas into dough by mixing. This has here been changed to "occlusion," etc., as suggested by the Editor.

Figure 1 gives the necessary information for reading either the percentage of gas by volume or the volume of gas in 100 g of dough, as determined by the method described above. If fats or high absorption are used in dough, the densities of gas-free dough are lowered; hence, Figure 1 will give slightly high indications of the amount of gas in such doughs. Low protein flours, on the other hand, give the opposite effect. Care is required in selecting the dough samples for this determination as large bubbles may accidentally be incorporated in the dough and give misleading figures. Also, doughs of low density, such as produced by overmixing, must be very carefully handled or gas will escape. Apparently such overmixed doughs contain gas in fairly large bubbles which are very easily eliminated by manipulation. When handling doughs containing yeast, it is necessary to determine the density as soon as the mixing is stopped.

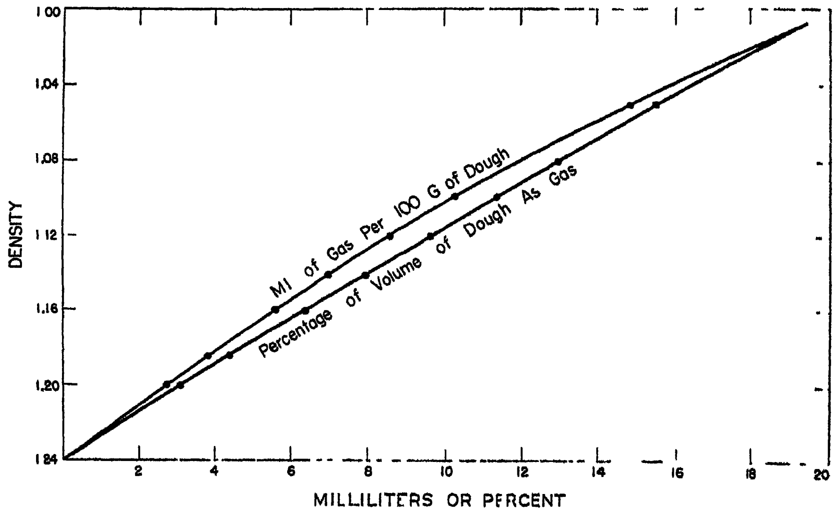


Fig. 1. The relation of dough density to the volume and percentage of occluded gas in average bread doughs without shortening.

Materials

Unless otherwise stated, four flours, Hard Spring Patent, Turkey, Chiefkan, and Durum, were used for the experiments. Two are of good and two of inferior breadmaking quality.

Doughs containing 340 g of flour, 2% salt, and 4% sugar were mixed at optimum absorption in carbon dioxide in the McDuffy bowl

TABLE I

CHEMICAL AND BAKING CHARACTERISTICS OF FOUR TYPES OF FLOURS

Flour	Protein	Ash	Mixing time	Absorption	Vol. of 1 lb loaf	Gluten puncture[1]
	(12.0% moisture)					
	%	%	*min*	%	*cc*	*g*
Hard spring patent	12.9	0.39	10	68	2830	29.4
Turkey	12.3	0.48	8	66	2715	25.4
Chiefkan (1941 crop)	12.7	0.40	3	67	2200	18.6
Durum	12.0	0.61	4	65	1800	16.0

[1] See Cereal Chem. 20: 506–516 (1943) for the method of determining gluten puncture.

at 65 rpm with the Hobart mixer. The optimum absorption of each flour was determined in a previous mix. To some doughs 40 ppm of sodium chlorite was added. Yeast was omitted in some cases to facilitate the density determinations. Its presence would not have materially altered the results unless testing was delayed. For experiments in the mixograph, doughs of the same relative composition formed from 35 g of flour were used. Carbon dioxide, which was released above the dough during mixing to eliminate the effects of oxygen, was retained by extending the mixograph bowl 1½″ upward with a thin sleeve.

Experimental

Dough Densities. Figure 2 gives the densities obtained by mixing the four flours (Table I) in the McDuffy bowl.

In doughs from the flours of good bread baking quality, gas dispersed very slowly both before and after the normal mixing requirement. The inferior flours occluded gas rapidly from the beginning of the mixing period. Much larger amounts of gas were occluded in the doughs made with these inferior flours, suggesting that excessive occlusion is not desirable and indicating that resistance to gas occlusion is one of the qualities of strong bread baking flours. This slower rate of occlusion of gas with the better bread flours could be interpreted to indicate that since such flours yield finer crumb which have more gas cells, the mixing causes an increase in the number of cells by gas subdivision rather than by occlusion. The resulting more numerous and smaller bubbles could be more readily surrounded by gluten and thus gastight bubbles be obtained, as suggested by Baker (1941).

Relation of Gas Occlusion to the Mixogram. Since gas occlusion is a foam-forming process, the changes encountered in dough mixing could be considered analogous to those in egg beating, with a similar stiffening and thinning taking place. This analogy suggested that the shape of

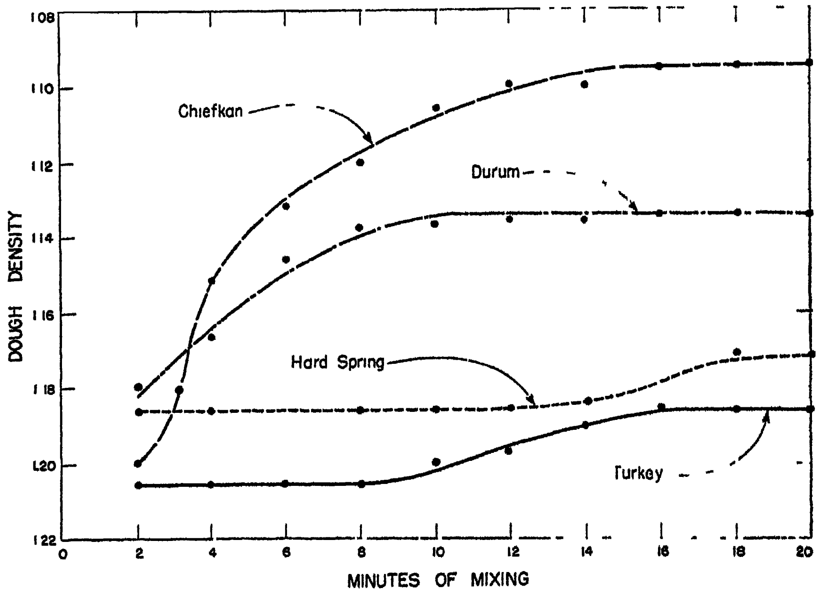


Fig. 2 Densities of unfermented, untreated doughs mixed in a McDuffy bowl in carbon dioxide.

a mixogram curve, due to changes in dough consistency, might result from gas occlusions. To test this hypothesis, densities were determined during the making of a mixogram. The relation between gas occlusion and dough consistency was thus observed.

The flours used in Figure 3 were the same as those listed in Table I. The densities of doughs were obtained during the course of the mixing operation of the mixograph by stopping the machine at a desired interval, removing a test sample, and determining the density. This procedure was repeated from the beginning on further samples of the same flour to determine other density values. Sufficient doughs were mixed to determine the densities throughout the entire mixogram. The densities of the doughs from each flour are plotted directly on the mixogram using the same time intervals on the abscissa but different units for density on the ordinate.

The results show that gas occlusion occurs more rapidly and somewhat more extensively in the mixograph than in the McDuffy bowl. The action in both mixers on different flours, however, is similar. The amount of gas occluded with time of mixing by these flours, with the exception of durum, is again in the reverse order of their bread baking quality, i.e., the more desirable bread baking flours show greater resistance to gas occlusion than do flours of poor baking quality.

The rate of gas occlusion increases slowly as the mobility curve first rises. When resistance to mixing reaches a maximum, the rate of occlusion greatly increases and gas is incorporated into the dough very rapidly. Thereafter gas occlusion again proceeds slowly. This continuing occlusion may be due, in part, to a rise in the temperature of the dough during mixing. The relative behavior of the mixogram and the gas occlusion curve suggests that the rise of the mixogram is due to gas occlusion. To further test this hypothesis, flours were mixed with 40 ppm sodium chlorite added to the liquor prior to combining with the flour, and the mixograms on the right side of Figure 3 were thus obtained. The oxidation caused a more rapid rise of the mixograms and a more rapid occlusion rate. The maximum rate of occlusion again coincides with the peak of the mixogram, suggesting, as before, that the rise in the pattern may be due to gas being incorporated in the dough.

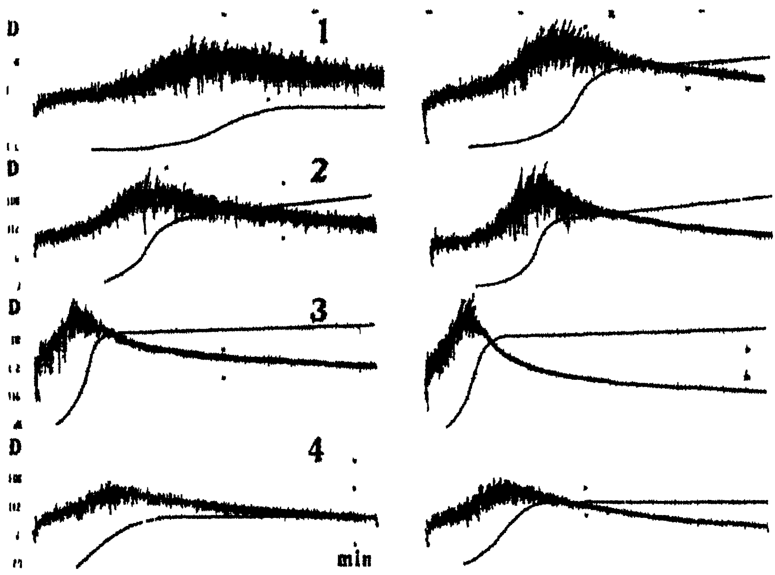
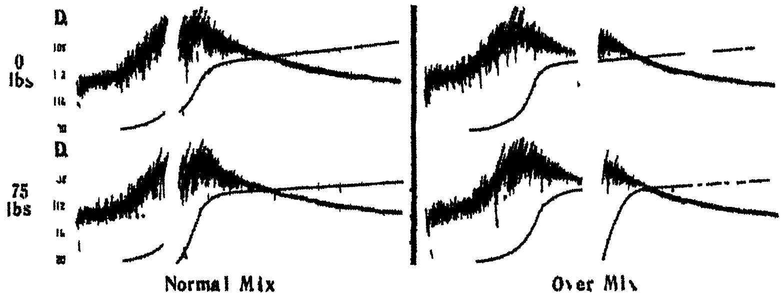


Fig. 3. Mixograms and densities of doughs made without (left side) and with sodium chlorite (right side).

If the increase in dough resistance as measured by the mixograph is due to occluded gas, then the resistance to mixing should decrease upon removing the occluded gas from the dough and increase again when the mixing is renewed. Most of the occluded carbon dioxide bubbles in dough can be collapsed and thus destroyed by subjecting the dough to pressure, thereby driving the gas into solution. If too much gas is present for accomplishing the desired collapse of the bubbles by pressure, it can be more completely eliminated by first placing the dough in a vacuum, then under pressure. The elimination of occluded gases is shown by a return of dough density to its initial value.

Figure 4 gives the results of these experiments on chlorite-treated doughs. The mixogram labeled "Normal Mix" and "75 pounds pressure" was interrupted at the point where optimum "no-dough-time" baking results were obtained (Baker and Mize, 1941). The bowl containing the dough was removed from the mixograph, subjected to vacuum and pressure as above described, then returned to the machine and the mixogram continued. The total interrupted time was 30 minutes. When a control dough labeled "0 lbs." was interrupted, but without pressure changes, the mixogram and density curve both continued as though no interruption had occurred. However, after the vacuum and pressure were applied, only the mixogram continued as though it had not been interrupted, whereas dough density was returned to its original value of 1.20. Upon the resumption of mixing the density dropped rapidly as gas occlusions were quickly renewed. Thereafter density changes were substantially the same as those of the uncompressed dough. These two experiments indicate that the rise in the mixogram is not due to occlusion of gas. To further test this point, the experiment was repeated on a dough interrupted after the peak of the mixogram, and after the rate of occlusion had passed its maximum. These results are also shown in Figure 4. Here again, the pressure affected the density only. It is now clear that occlusion is only an accompanying phenomenon of mixing. The thickening or thinning of the dough during mixing is not due to occluded gas. The occlusion of gas always increases with the rise in the mixogram, and is fastest at its peak, after which further occlusion



Normal Mix

Over Mix

Fig. 4. Mixograms and densities obtained when vacuum and pressure were applied to doughs from interrupted mixograms

continues slowly. Thus, apparently, the phenomenon causing the mixogram to rise to a peak also causes the dough to occlude gas. This coincidence of gas being trapped in dough at the time of maximum resistance to mixing suggests that in the formation of gluten during mixing, bubbles are caught in the connected gluten micelles as they cohere.

Relation of Gas Occlusion to Baking. Baking experiments using the "no-dough-time" technique have indicated that the best bread is obtained just before the rapid rate of gas occlusion occurs. To test this relationship micro-loaves were mixed in the mixograph and baked by a technique similar to that described by Van Scoyk (1937, 1939), but modified to the "no-dough-time" method using 40 ppm sodium chlorite, described by Baker and Mize (1941). The operation of the mixograph was stopped, the dough removed and immediately panned, proofed, and baked. Five doughs were mixed to the following degrees, respectively: the minimum (1½ minute mix), the optimum baking value (4¼ minute mix), the peak of the mixogram (5 minute mix), beyond the peak (5½ minute mix), and badly overmixed (12 minute mix). The mixogram, the densities, and the loaves from these five doughs are given in Figure 5.

The minimum mixed loaf indicates a lack of sufficient occluded gas nuclei to yield a fine cellular structure; hence, the very coarse grain.

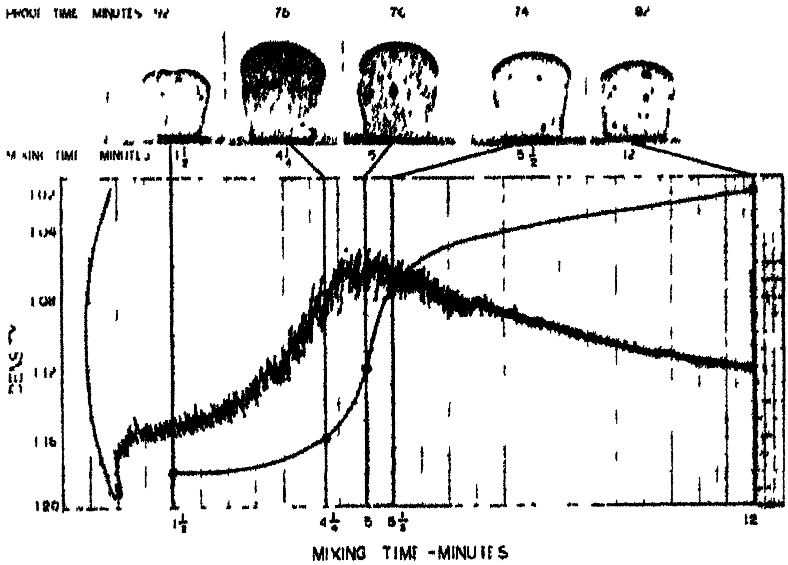


Fig. 5. Micro-loaves panned at once and baked from mixogram doughs at successive intervals of mixing with 40 ppm sodium chlorite.

Further, its long proof time and small volume indicate that the cells present failed to retain gases sufficiently to give normal proof or oven spring. The optimum loaf shows large volume with very fine uniform grain and absence of large bubbles. This loaf was taken just prior to the sharp decrease in density which occurs at the peak of the mixogram, and shows that at this point ample gas has been occluded for breadmaking and that it is divided into many gastight cells of small size. The loaf baked at the peak of the mixogram with 5 minutes of mixing shows the presence of large bubbles dispersed among the finely divided cells observed in the previous loaf. This cell development suggests that the gas rapidly occluded in the additional one-half minute to the peak of the mixogram was not all divided into small cells of uniform size. The larger cells expand during proofing but do not coalesce or rise through the dough because of the highly oxidized nonfluid character of these "no-dough-time" doughs, as reported by Baker, Parker, and Mize (1942). This loaf illustrates why the mixing period in making "no-dough-time" doughs is very critical. It has always been observed that the mixing must be stopped at a definite interval of time to obtain optimum results, and that further mixing results in irregular grain and cell size.

The remaining loaves show marked loss in volume from mixing beyond the mixogram peak. The one which was mixed 5½ minutes has very fine grain in addition to some large bubbles, indicating that some of the gas occluded during the peak of the mixing curve had been further subdivided. These overmixed doughs fail to retain gas during oven spring. The reason for this loss of volume must be due either to leakage of gas from the cells or to coalescence. The fine crumb of the 5½ minute mixed loaf shows that the gas cells did not coalesce, and the location, shape, and size of the large cells indicate that they did not move upward in the dough. Hence, the failure of this dough to spring fully in baking must be due to cell leakage. The badly overmixed dough, at 12 minutes of operation, became so fluid, as shown by its lowered resistance, that most of the gas cells suggested by its low density were lost because of coalescence and upward movement of the enlarged bubbles. The character of this badly overmixed dough can also be explained by excessive cell leakage as only a few bubbles retained gas sufficiently to expand. This is also indicated by the longer proof time. The drop in the mixogram pattern of the two overmixed doughs indicates a loss of strain in the doughs and suggests disintegration of the gluten complex to decrease its continuity which permits leakage and finally coalescence.

To show the effects of mixing without oxidation, the above experiments in baking bread at different stages of the mixing period were repeated with doughs mixed in the McDuffy bowl. The undermixed, optimum-mixed, and overmixed doughs only were prepared because it is difficult when operating the McDuffy bowl to pick that point in the mixing which corresponds exactly with the peak of the mixogram.

Figure 6 gives two sets of three loaves each mixed in this manner. Both sets were made by the "no-dough-time" technique, one being oxidized with 40 ppm sodium chlorite, the other without oxidation. The breads made from oxidized doughs, when mixed in different amounts in the McDuffy bowl, show the same characteristics in grain and have similar dough density to the corresponding mixograph doughs, and thus suggest the number of cell nuclei observed is similar to the loaves mixed in the mixograph. When no sodium chlorite was used, identical loaves of very green character were obtained with all amounts of mixing. The densities of the unoxidized doughs, however, were substantially the same as those of the oxidized doughs at the different stages of mixing. The unoxidized bread fails to show any evidence of the gas occlusions indicated by the densities. The cells formed in the mixing must have disappeared from the dough during the proofing and baking of the bread by coalescence and upward and outward movement.

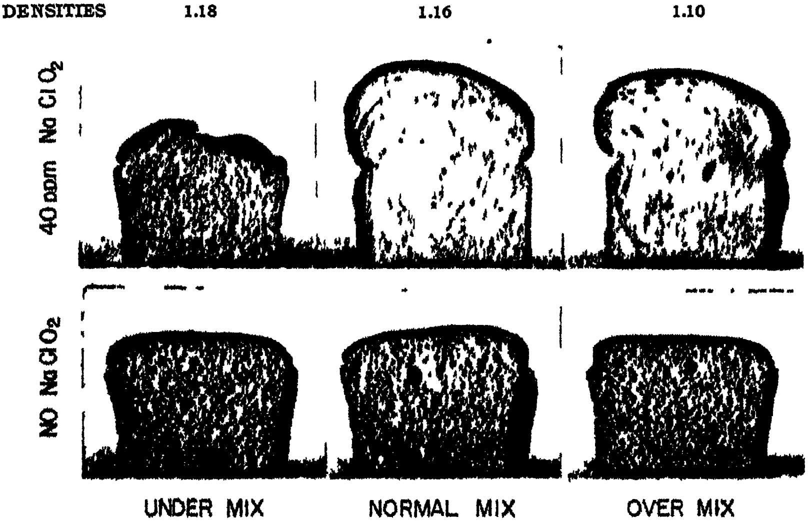


Fig. 6. "No-dough-time" doughs with and without sodium chlorite panned at once after different periods of mixing in a McDuffy bowl.

Relaxation of Doughs. In order to explain the apparent difference in the oxidized and unoxidized "no-dough-time" breads of Figure 6, similar doughs were formed in the mixograph. The operation of the machine was interrupted at stated intervals for a period of one-half hour, after which the operation of the machine was resumed to continue the mixogram.

Densities of the doughs for all points determined were obtained by repeating the operation of the mixograph on separate doughs. These densities are plotted on the corresponding interrupted mixograms in Figure 7. The interruption of the mixograms in these two series was made when the amount of mixing was equivalent to that used in forming the doughs of the breads in Figure 6. The equivalent mixing times of the two mixers were determined by separate baking experiments. It is to be noted that no occluded gas was lost during the interruption of mixing. Also, with one exception, the march of densities of the doughs continued upon resumption of mixing as though no interruption had occurred. The exception was in the unoxidized normal mix where the dough had reached the condition of rapid occlusion. This condition was lost during the rest period but was regained as the dough recovered its resistance to mixing. The mixograms of Figure 7 give an explanation for the marked difference between the "no-dough-time" breads of Figure 6 from doughs with and without sodium chlorite. In both series, the doughs resisted mixing and occluded gas in approximately the same characteristic manner, indicating organization of the gluten. Upon interruption, the doughs without chlorite were unable to retain the strain produced in the dough by the mixer. During the one-half hour standing these unoxidized doughs relaxed without losing occluded gas. Their resistance upon resumption of mixing was approximately the same as at the initial period of the mixing. The oxidized doughs, on the other hand, retained the mixing strain throughout the one-half hour standing and the mixogram resumed, in the case of normal or optimum mix, substantially as though the interruption had not taken place. Un-

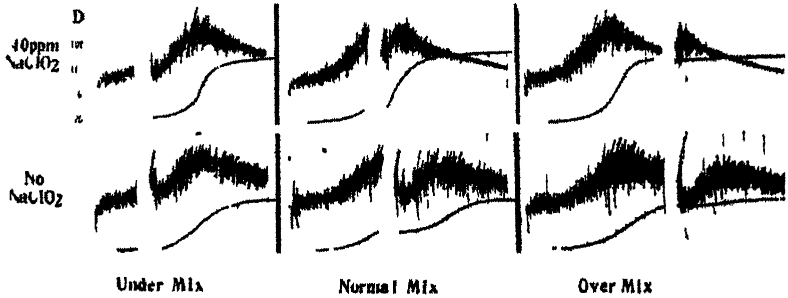


Fig. 7. Interrupted mixograms showing changes in dough mobility and density while resting. Doughs were similar to those used for Figure 6.

doubtedly, the same property that causes the oxidized dough to retain mixing strain during standing also causes it to retain its occluded gas and cell structure during proofing and baking. Flow is substantially prevented; hence, coalescence or cell movement does not occur because the strain is not released. In the unoxidized dough the bubbles as they expand are unable to retain the strain, the dough structure relaxes, the cells coalesce and move through the dough; hence, the initial cell content created in the mixing is lost in the proof. This interpretation was also demonstrated in dough freezing experiments by Baker and Mize (1941). These experiments show that the effects of oxidation in breadmaking are obtained by reactions that retain the strains developed in dough while it is being worked.

Density of Sponge Doughs. The "no-dough-time" method of making bread used for all the work so far described herein is a useful tool for research. It differs from methods employed in practice in that all operations except mixing, proofing, and baking are eliminated. Further, it relies upon mixing to initiate all gas cells. It permits a minimum of enzymatic changes and fermentation products during the entire operation. The interpretations obtained from these "no-dough-time" breads cannot necessarily be applied to other methods of breadmaking. To study the mixing effects obtained with other methods of operation, the densities of commercial doughs were studied with the collaboration of four commercial bakeries. The results are listed in Table II.

TABLE II

DENSITIES OF COMMERCIAL SPONGE DOUGHS TAKEN AT THE END OF THE FINAL MIX[1]

Bakery	Dough density
A	1.130 (Average on 8 doughs) (With range 1.121 to 1.135)
B	1.130
C	1.115
D	1.120

[1] The authors wish to take this opportunity to express their thanks to Messrs. Gaston Dalby, J. H. Lanning, W. H. Cathcart, and R. I. Bohn for their collaboration in obtaining the densities on commercial doughs.

These values are considerably lower than any densities reported for optimum mixed "no-dough-time" doughs. To examine the reasons for this difference a comparative study was made of the dough density obtained in the McDuffy bowl with a 60/40 sponge dough and the density of an unfermented dough from the same flour.

The mixing of an unfermented dough with or without bromate in a McDuffy bowl gives the dough density results shown at the bottom of Figure 8. This density curve is similar to that obtained on this

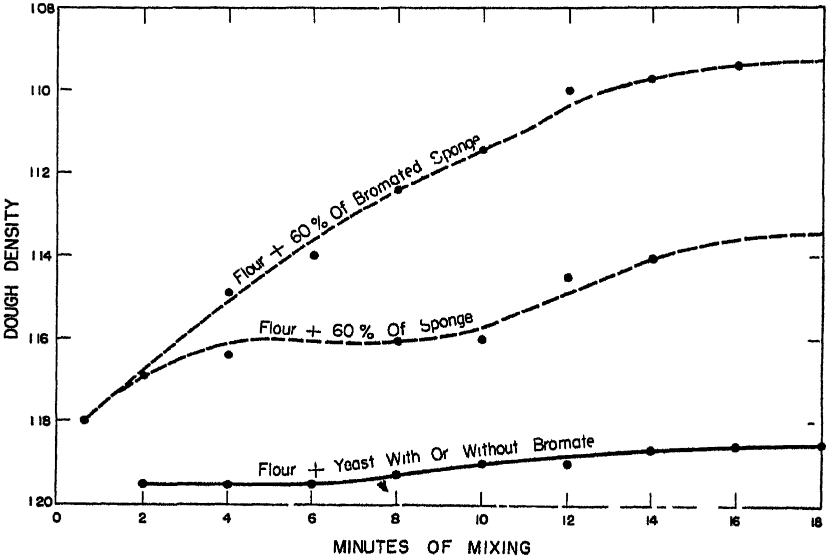


Fig 8. Density during mixing of unoxidized and oxidized doughs formed from water, salt, and either flour and yeast, or flour and 60% sponge

strong spring wheat patent flour shown as number 1 in Figure 2. It will be observed that bromate is without effect on dough density while assembling an unfermented dough. When undermixed doughs, as in a sponge, are permitted to ferment and then incorporated together with 40% new flour into a sponge dough, much greater occlusion of gas is obtained during mixing. Without bromate a density of 1.16 was obtained by mixing such dough to the optimum. When bromated sponge was mixed with the 40% new flour, a density of 1.125 was obtained by mixing to the optimum. This latter value is in the range of densities observed in commercial sponge doughs. These results indicate that fermentation markedly increases the rate of incorporation and amount of gas which dough occludes during mixing. Also, these results show that bromate increases the gas occlusion capacity of fermented doughs in a manner similar to the lesser action of sodium chlorite on unfermented doughs.

Summary

The amount of gas present in dough can be determined by comparing its density to that of a similar gas-free dough. Flours vary widely in the amounts of gas which they occlude during mixing. The slower mixing and better bread flours are more resistant. The rate at which gas is occluded during mixing varies widely during different stages of the mixing period, being slow at first and fastest at the time the dough offers its greatest resistance to mixing. Dough densities plotted on a mixogram at successive time intervals suggest that the resistance to mixing is caused by the occluded gas. This was shown not to be true by removing occlusions of carbon dioxide gas by means of vacuum and pressure. With dough oxidized by means of sodium chlorite no change in the mixing resistance curve was produced by thus removing the occluded gas.

Undermixed doughs contain the smallest amount of occluded gas. Baking experiments with mixograph doughs show that in undermixed doughs the number of gas cells is small and they are leaky. This is shown by the high density, the coarse grain, small volume, and long proof time. The best bread is obtained near the peak of the mixogram just before the rapid rate of gas occlusion is reached. This fact, together with the fine grain of the bread, its large volume, and short proof time, indicates that the fine cell structure is obtained, not by gas occlusion, but by gas subdivision, and by organization of the dough and gluten, so the cells are gastight and retain their integrity throughout the breadmaking process. Further mixing rapidly incorporates gas to yield irregular-sized bubbles and nonuniform grain. Excessive overmixing results in loss of gluten continuity and coherence so that few cells are sufficiently gastight to expand and form the visible cell structure of bread.

The action of oxidation in improving doughs is not due to effects on gas occlusion but rather to preventing doughs from relaxing or flowing. Hence, oxidation of doughs prevents coalescence and bubble movement and results in retention of the occluded gas cells during their expansion in breadmaking.

Fermentation greatly increases the gas occlusion rate and capacity of doughs. Bromate further increases this effect. Commercial type sponge doughs mixed in a McDuffy bowl gave optimum doughs of the same density as a number of commercial doughs which were tested.

Literature Cited

Baker, J. C.
1941 The structure of the gas cell in bread dough. Cereal Chem. 18: 34 41.
—— and Mize, M. D.
1937 Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. 14: 721-734.
1941 The origin of the gas cell in bread dough. Cereal Chem. 18: 19–34.
——, ——, and Parker, H. K.
1943 Measurement and significance of gluten quality. Cereal Chem. 20: 506–516.
——, Parker, H. K., and Mize, M. D.
1942 The action of an oxidizing agent in bread dough made from patent flours. Cereal Chem. 19: 334–346.
Van Scoyk, W. V.
1937 A molder for micro-baking. Cereal Chem. 14: 263–265.
1939 Micro-baking technique, applications and results. Cereal Chem. 16: 1–12.

EFFECT OF LEAF AND STEM RUST ON THE PHOSPHORUS METABOLISM OF HARD RED SPRING WHEAT[1]

A. Hoffer,[2] W. F. Geddes,[3] and M. N. Levine[4]

(Received for publication April 18, 1945)

One of the objects of a cereal rust research program conducted at the University of Minnesota was to study the effect of leaf and stem rust upon the translocation of certain plant constituents into the developing kernel and on the chemical composition of the resulting grain. Geddes and Levine (1942) have reported the results of a study of the distribution of thiamine in Thatcher wheat during ripening in 1940. A similar investigation into the phosphorus content and its distribution in Thatcher and Marquis wheat, and the effect of rust thereon in 1941, is reported in this paper.

The literature on the phosphorus metabolism of wheat has been adequately reviewed by Miller (1939) in connection with his extensive studies on the chemical composition of the wheat plant at different stages of its development. Although several workers have studied the concentration of phosphorus, phosphorus content, and its distribution in the wheat plant at various stages of maturity, there is only limited information on the influence of rust infection on the phosphorus metabolism.

Most investigators have found that the actual amount of phosphorus present in the stems, leaves, and glumes decreases during the maturation of the plant; whereas the quantity of phosphorus present in the kernel increases. There is, however, lack of agreement on the trends in the total phosphorus content of the entire plant as it matures. Some investigators have found that the total amount of phosphorus in the plant reaches a maximum about the time of blossoming and thereafter decreases, presumably due to the return of phosphorus to the soil. Others report that some absorption of phosphorus occurs during its translocation into the grain, while still others have noted that the total phosphorus content of the plant remains relatively stationary from blossoming to maturity.

In his extensive studies in Kansas of the changes in dry matter content and chemical composition of rust-free hard winter and soft winter wheat from the seedling stage to complete maturity, Miller (1939) found that phosphorus continued to be absorbed from the soil after heading and that the total amount of phosphorus in the plant did not generally reach a maximum until harvest. In general, the amount of phosphorus in the stems and leaves decreased, and that in the head increased, from the time of heading until full maturity.

---

[1] Joint contribution from the Division of Agricultural Biochemistry and the Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture. Paper No. 2254. Grateful acknowledgment is made for valuable assistance rendered by the personnel of the Work Projects Administration, Official Project No. 165-1-71-124, sponsored by the University of Minnesota during the year 1941.

[2] Research Chemist, Western Canada Flour Mills Company, Ltd., Winnipeg, Manitoba.

[3] Professor and Chief, Division of Agricultural Biochemistry, Department of Agriculture, University of Minnesota.

[4] Pathologist, Division of Cereal Crops and Diseases, United States Department of Agriculture, detailed for cooperative research work to the Division of Plant Pathology and Botany, University of Minnesota.

Caldwell, Kraybill, Sullivan, and Compton (1934) studied the effect of leaf rust infection on the yield, physical characteristics, and chemical composition of winter wheat. Phosphorus determinations were made only on the grain itself at successive stages of development. They found that leaf rust produced no significant changes in the percentage of phosphorus present in either immature or mature grain. However, as severe rust infection reduced the head yield and acre yield, the total quantities of phosphorus per head were greater in the mature control plants.

Greaney, Woodward, and Whiteside (1941) studied the effect of stem rust on the productivity, chemical composition, and quality of mature Marquis, a hard red spring wheat variety. Like Caldwell *et al.* they found no significant difference in the percentage of phosphorus in the grain as a result of severe stem rust infection in 1934 and 1935. In 1937, however, the percentage of phosphorus in the grain appeared to increase with the increase in severity of stem rust infection. In their experiments, appreciable leaf rust infection occurred along with stem rust in the 1934 and 1935 series. The authors suggest that in the grain, "rust infection interferes considerably more with the deposition of organic than with inorganic compounds." In 1937, analyses were made of the mature straw in addition to the ripe grain. Severe rust infection resulted in a 50% increase in the percentage of phosphorus in the straw, in comparison with the relatively rust-free plants.

Materials and Methods

In the present investigation, Thatcher was used for the study of the effect of leaf rust, Marquis for that of stem rust. Both varieties were sown on May 5, plot treatments were started May 19, and continued through July 21. Inoculations with a uredial composite of 16 physiologic races of *Puccinia rubigovera tritici* (Erikss. and Henn.) Carl. prevalent in the spring wheat region were used to induce the leaf rust epidemic; a composite of 14 common physiologic races of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. was used to induce the stem rust epidemic.

Relative control and regulation of the intensity of the rust epidemics were obtained by means of sulfur dusting. Two methods of approach, "preventive" and "inhibitive," were employed. By the use of the preventive method, the incidence of rust was delayed by sulfur dusting until certain stages in plant development were reached; after this, artificial rust inoculations were made at weekly intervals. In the inhibitive series, rust infection was induced artificially in the early stages of plant growth; further progress of the epidemics was arrested at given periods by weekly applications of sulfur dust.

The samples used in this study were limited to plants taken from two sets of replicate test plots, eight of which represented the lightest, and eight, the severest, rust infection that prevailed. In the least severely infected plots, no artificial inoculations were made and natural rust incidence was controlled by weekly applications of sulfur dust. In the most severely infected plots, the plants were inoculated with rust at weekly intervals but received no sulfur dusting. The first samples for the biochemical determinations were cut from the Thatcher plots on July 7, those from the Marquis plots, a week later; subsequent samples were obtained at semiweekly intervals thereafter. The collection and preparation of the samples for analyses followed the methods described by Geddes and Levine (1942).

As an index of progressing maturity, the dry matter weights per thousand kernels and the moisture contents of the entire wheat tiller were determined. Five hundred air-dry kernels per sample were counted and weighed in duplicate. The moisture contents of each plant fraction were determined on samples ground in a Wiley laboratory mill (equipped with a sieve having 0.5 mm openings) by the one-hour, 130°C air-oven procedure, as described in *Cereal Laboratory Methods* (4th ed., 1941). The moisture contents of the entire tillers were computed from the original weights of the various fractions and the moisture lost on air-drying and on oven-drying.

The samples were analyzed in duplicate on different days for total phosphorus by the colorimetric procedure, outlined in *Cereal Laboratory Methods* (4th ed.). Transmission of light was determined in a Coleman Model 11 Universal Spectrophotometer at 533 mμ. The ash content of the samples was determined by ignition overnight at approximately 575°C. The phosphorus (expressed as P) and ash results were computed on the dry matter basis.

Results and Discussion

The data for kernel weights and moisture contents of the entire tillers at successive stages of maturity of Thatcher and Marquis wheat variously infected with leaf and stem rust, respectively, are presented in Table I. The percentages of phosphorus present in the three fractions—grain (kernels), chaff (glumes and rachides), and straw (stems and leaves)—into which the tillers were divided, and the percentage of phosphorus in the entire tillers, are recorded in Table II. Since the weights of each fraction and the number of tillers represented were known, it was possible to calculate the total quantities of phosphorus present in each fraction and in the entire tiller. These data are given in Table III. The percentage distribution of the total phosphorus of the tiller among the three plant fractions was computed from the data of Table III and are recorded in Table IV.

Effect of Leaf and Stem Rust on Kernel Weight and Plant Transpiration. The effect of severe leaf rust infection on the weight of the developing Thatcher kernels became pronounced 3 weeks after blossoming, while the effect of stem rust on Marquis became evident a week sooner. The ultimate decrease in kernel weight of Thatcher due to leaf rust was 2.5 g per 1,000 kernels, as compared with a decrease of 6.2 g per 1,000 kernels for Marquis on account of stem rust (Table I). The increased rate of moisture loss brought about by

TABLE I

Effect of Maturity and Rust Infection on the Weight per Thousand Kernels and the Moisture Content of the Tillers

Days after blossoming	Successive stages of kernel development	Most lightly rusted wheat		Most severely rusted wheat	
		Weight per 1,000 kernels (dm basis)	Moisture content of aerial tiller	Weight per 1,000 kernels (dm basis)	Moisture content of aerial tiller
		g	%	g	%
Thatcher		31.7% leaf rust		89.5% leaf rust	
7	Pre-milk	3.7	70.8	4.6	71.2
10	Early milk	6.0	69.8	6.6	68.3
14	Late milk	9.1	65.2	9.7	65.1
17	Soft dough	12.5	64.0	12.5	62.6
21	Medium dough	16.1	59.0	16.5	57.2
24	Hard dough	19.9	52.2	18.1	48.8
28	Semiripe	20.1	39.6	17.7	26.2
31	Full ripe	21.2	25.8	18.7	20.8
Marquis		3.2% stem rust		48.6% stem rust	
7	Pre-milk	6.0	68.4	5.9	67.3
10	Early milk	9.9	66.0	9.7	65.6
14	Late milk	14.2	61.2	13.9	59.7
17	Soft dough	18.9	54.4	15.0	52.1
21	Medium dough	23.3	46.8	16.9	35.4
24	Hard dough	24.0	36.2	17.5	20.5
29	Near ripe	24.4	22.6	18.2	19.3

severe leaf rust infection became quite evident 24 days after blossoming and reached its peak 4 days later; in the case of stem rust, the greater moisture loss of the severely infected plants became pronounced 21 days after blossoming and reached its maximum 3 days later. Even though the average maximum stem rust infection on Marquis was only 48.6%, as compared with an average maximum of 89.5% leaf rust on Thatcher, the effects on average kernel weight and moisture content of the tiller were sharper in the case of stem rust.

Effect of Maturity on Phosphorus Content and Its Distribution. The results for the least severely infected samples of Thatcher (average 31.7% leaf rust) and of Marquis (average 3.2% stem rust) are probably the most indicative of the normal course of phosphorus metabolism and, therefore, the discussion of the effect of maturity on phosphorus content and its distribution will be based principally on the data obtained from the most lightly infected series of test plots. However, similar trends were exhibited by the results procured in the most severely rusted series.

TABLE II

EFFECT OF MATURITY AND RUST INFECTION ON THE PHOSPHORUS CONCENTRATION IN VARIOUS FRACTIONS OF THE WHEAT PLANT

Days after blossoming	Successive stages of kernel development	Per cent phosphorus (P)—dry matter basis							
		Most lightly rusted wheat				Most severely rusted wheat			
		Grain	Chaff	Straw	Entire aerial tiller	Grain	Chaff	Straw	Entire aerial tiller
		%	%	%	%	%	%	%	%
Thatcher		31.7% leaf rust				89.5% leaf rust			
7	Pre-milk	0.59	0.30	0.26	0.28	0.62	0.30	0.29	0.31
10	Early milk	0.53	0.31	0.23	0.28	0.61	0.26	0.27	0.31
14	Late milk	0.51	0.25	0.22	0.28	0.56	0.23	0.23	0.31
17	Soft dough	0.54	0.23	0.18	0.28	0.54	0.24	0.21	0.30
21	Medium dough	0.49	0.24	0.16	0.26	0.55	0.21	0.17	0.31
24	Hard dough	0.51	0.22	0.12	0.28	0.53	0.24	0.15	0.29
28	Semiripe	0.56	0.24	0.10	0.28	0.54	0.25	0.13	0.28
31	Dead ripe	0.55	0.24	0.09	0.29	0.56	0.25	0.13	0.29
Marquis		3.2% stem rust				48.6% stem rust			
7	Pre-milk	0.51	0.31	0.27	0.29	0.60	0.32	0.28	0.31
10	Early milk	0.56	0.28	0.21	0.27	0.53	0.31	0.27	0.31
14	Late milk	0.53	0.29	0.19	0.28	0.55	0.28	0.24	0.31
17	Soft dough	0.55	0.26	0.17	0.29	0.56	0.31	0.19	0.31
21	Medium dough	0.53	0.24	0.09	0.25	0.56	0.33	0.18	0.31
24	Hard dough	0.57	0.23	0.09	0.28	0.57	0.26	0.15	0.29
29	Near ripe	0.56	0.20	0.08	0.27	0.59	0.14	0.12	0.27

In both Thatcher and Marquis the percentage of phosphorus in the grain showed no definite trends as maturity progressed (Table II). In the chaff the percentage of phosphorus tended to decrease slightly as the plants approached maturity. In the straw the percentage of phosphorus decreased greatly with maturity, constituting in the ripe plants only one-third of what it was at one week after flowering. In the entire tiller the concentration seemingly remained relatively constant.

When comparisons are made on the basis of total phosphorus per 100 tillers of lightly rusted plants (Table III), the phosphorus content

TABLE III

EFFECT OF MATURITY AND RUST INFECTION ON THE PHOSPHORUS CONTENT OF VARIOUS FRACTIONS OF THE WHEAT PLANT

Days after blossoming	Successive stages of kernel development	Phosphorus content (P) per 100 tillers							
		Most lightly rusted wheat				Most severely rusted wheat			
		Grain	Chaff	Straw	Entire aerial tiller	Grain	Chaff	Straw	Entire aerial tiller
		g	g	g	g	g	g	g	g
Thatcher		31.7% leaf rust				89.5% leaf rust			
7	Pre-milk	0.03	0.08	0.28	0.39	0.05	0.08	0.27	0.39
10	Early milk	0.08	0.08	0.22	0.38	0.09	0.06	0.23	0.38
14	Late milk	0.14	0.06	0.23	0.44	0.13	0.05	0.17	0.35
17	Soft dough	0.20	0.05	0.16	0.41	0.17	0.05	0.16	0.38
21	Medium dough	0.24	0.06	0.13	0.43	0.24	0.05	0.13	0.42
24	Hard dough	0.29	0.05	0.10	0.44	0.23	0.05	0.10	0.37
28	Semiripe	0.28	0.05	0.07	0.40	0.21	0.04	0.08	0.33
31	Dead ripe	0.34	0.06	0.06	0.46	0.21	0.05	0.08	0.34
Marquis		3.2% stem rust				48.6% stem rust			
7	Pre-milk	0.07	0.11	0.35	0.52	0.09	0.10	0.35	0.54
10	Early milk	0.14	0.09	0.26	0.49	0.13	0.09	0.31	0.53
14	Late milk	0.20	0.08	0.21	0.49	0.22	0.08	0.26	0.56
17	Soft dough	0.29	0.07	0.18	0.55	0.23	0.08	0.19	0.50
21	Medium dough	0.33	0.07	0.09	0.49	0.24	0.08	0.16	0.49
24	Hard dough	0.40	0.07	0.09	0.56	0.29	0.07	0.14	0.49
29	Near ripe	0.39	0.06	0.08	0.53	0.31	0.05	0.11	0.47

of the grain of both varieties increased greatly until mature, over tenfold in the case of Thatcher and about sixfold in that of Marquis. The total phosphorus content of the chaff decreased to two-thirds of its original value in Thatcher and to approximately one-half in Marquis. In the straw the total phosphorus of both varieties decreased to less than one-quarter of the amount present one week after flowering. In the entire tiller no clear-cut change was apparent, considering the fact that errors in determining phosphorus and dry matter are accumulated in calculating the values. The data seem to indicate that most, if not all, of the phosphorus ultimately found in the mature plant tiller is present within a week or two after blossoming and thereafter is translocated from the chaff and straw into the developing kernels.

The translocation of phosphorus into the kernels is clearly demonstrated by the changes in the percentage distribution of the total phosphorus in the three plant fractions as the plants mature (Table IV).

TABLE IV

Effect of Maturity and Rust Infection on the Distribution of Phosphorus in the Wheat Plant

Days after blossoming	Successive stages of kernel development	Distribution of phosphorus					
		Most lightly rusted wheat			Most severely rusted wheat		
		Grain	Chaff	Straw	Grain	Chaff	Straw
		%	%	%	%	%	%
Thatcher		31.7% leaf rust			89.5% leaf rust		
7	Pre-milk	8.7	19.5	71.8	11.9	20.1	68.0
10	Early milk	21.4	20.6	58.0	23.6	15.1	61.3
14	Late milk	32.9	14.1	53.0	36.9	13.6	49.5
17	Soft dough	48.3	12.9	38.8	44.0	13.0	43.0
21	Medium dough	56.3	12.8	30.9	57.4	11.2	31.4
24	Hard dough	67.2	11.1	21.7	61.1	12.8	26.1
28	Semiripe	70.2	12.2	17.6	63.0	13.2	23.8
31	Dead ripe	73.9	12.2	13.9	61.5	15.0	23.5
Marquis		3.2% stem rust			48.6% stem rust		
7	Pre-milk	12.8	20.6	66.6	17.3	19.1	63.6
10	Early milk	28.1	18.7	53.2	24.6	17.3	58.1
14	Late milk	41.7	16.4	41.9	39.5	14.8	45.7
17	Soft dough	53.0	13.4	33.6	46.7	16.0	37.3
21	Medium dough	68.1	13.3	18.6	49.9	16.6	33.5
24	Hard dough	71.9	12.1	16.0	58.5	13.9	27.6
29	Near ripe	74.2	10.8	15.0	66.3	9.6	24.1

In the lightly rusted Thatcher the percentage of the total phosphorus present of the entire tiller increased in the kernels from 8.7% a week after flowering to 73.9% at full maturity; in the chaff it decreased from 19.5% to 12.2%; and in the straw it was reduced from 71.8% to 13.9%. Similar translocation trends were evident in the case of lightly rusted Marquis.

It is of interest to compare these data on hard red spring wheats grown in Minnesota with the corresponding study conducted by Miller (1939) with winter wheats grown in Kansas. In contrast to our results, he found, with only one exception, that the total amounts of phosphorus in the plants reached their maximum around harvest time However, he found very similar trends to those reported here—in the distribution of the total phosphorus in the various plant fractions, as maturity progressed. As the kernels developed, the total phosphorus in the head increased, and that in the stems and leaves decreased. Similar observations on the translocation of phosphorus during kernel development have been made by many other workers, as reviewed by Miller. In agreement with our data, he found that the percentage of phosphorus present in the stems and leaves decreased from head formation to harvest In two of the three years in which his studies were conducted, he found that the percentage of phosphorus in the heads, in general, showed a slight decrease from the time of their formation to maturity; in the third year, the percentage of phosphorus showed a marked decrease for four weeks and then slightly increased during the final two weeks of maturity. The percentage of phosphorus in the chaff also markedly decreased from head formation to harvest In our studies, there were no consistent trends in the percentage of phosphorus in the kernels as the grain matured, but the concentration in the glumes and rachides decreased appreciably.

The similarity in the translocation of phosphorus found in this study with that of thiamine, as reported by Geddes and Levine (1942), is very striking. However, the initial proportion of the total thiamine present in the kernels was higher, and the final percentage in the glumes and rachides was much lower than the corresponding values for phosphorus.

Effect of Rust Infection on Phosphorus Content and Its Distribution. A comparison of the results obtained for the most severely infected samples of Thatcher (average 89.5% leaf rust) and of Marquis (average 48.6% stem rust) with the data from the least severely rusted plots (Thatcher, average of 31.7% leaf rust; Marquis, average of 3.2% stem rust) provides a measure of the effect of rust on the metabolism of the wheat plant.

During the early part of the ripening period, the kernels of the severely rusted spring wheats contained a slightly higher percentage of phosphorus than the kernels of the lightly rusted wheats; these differences decreased with maturity (Table II). These results are in general agreement with those obtained by Caldwell *et al.* (1934), who found that leaf rust produced no significant changes in the percentage phosphorus present in the grain of winter wheat at various stages in its development. Greaney *et al.* (1941) also found no differences in the percentage of phosphorus present in the mature kernels of Marquis wheat, variously affected by stem rust, in two of the three years in which their studies were carried out. In the present study the percentage of phosphorus in the chaff was not affected, in any definite manner, by severe leaf rust infection during any stage of kernel development; but severe stem rust infection tended to result, though not entirely consistently, in a slightly higher percentage of phosphorus in the chaff at the more advanced stages of maturity. However, regardless of the rust involved, the stems and leaves of the heavily infected plants contained a higher percentage of phosphorus than the lightly infected plants through the entire period of kernel development. At maturity the percentage of phosphorus in the straw of plants severely infected with either leaf or stem rust was approximately 50% greater than in that of the lightly infected plants. These results parallel closely the results obtained by Greaney *et al.* (1941) on the effect of stem rust on the percentage of phosphorus in the straw. In the entire tillers the percentage of phosphorus was slightly higher in the heavily rusted plants during the early stages of kernel development; but in the fully ripe plant the differences completely disappeared.

In contrast, the total phosphorus content of the entire tiller was markedly lowered by severe rust infection 24 days after blossoming, while its translocation into the kernel was arrested concurrently in the case of leaf rust, and a week earlier in the case of stem rust (Table III). The decrease in total phosphorus is associated with the effect of cereal rust on yield of dry matter, whereas the interference with translocation is associated with the more rapid desiccation of the severely infected plants, due to excessive transpiration.

In the early stages of kernel development an appreciably higher percentage of the total phosphorus was present in the kernels of the most severely infected Thatcher and Marquis than in those of the least severely infected plants (Table IV). Thus, a week after flowering, the most severely rusted Thatcher kernels contained 11.9%, whereas the kernels of the most lightly infected Thatcher contained only 8.7% of the total phosphorus. The corresponding values for Marquis were 17.3% and 12.8%, respectively. However, the mature kernels of the more lightly leaf rust-infected Thatcher contained 73.9% of the total phosphorus of the entire tiller, whereas, in the most severely affected plants, the ripe kernels contained only 61.5% of the total phosphorus. With Marquis, on which the average maximum stem rust infection (48.6%) was much lower than the average maximum leaf rust infection on Thatcher (89.5%), the effect of the rust on translocation was somewhat less pronounced; the mature Marquis kernels from the most lightly infected plants contained 74.2% and those from the most severely rusted plants contained 66.3% of the total phosphorus. It is evident from the results obtained in this study that the over-all rate and extent of translocation is markedly influenced by the severity of rust infection. Thus, the difference between the final and initial percentages of the total phosphorus present in the kernels in the most lightly leaf rust-infected Thatcher was 65.2%, as compared with a difference of 49.6% for the most severely rusted Thatcher. The corresponding differences for stem rust-infected Marquis were 61.4% and 49.0%, respectively. The retarding rate of translocation in the severely rusted plants became most pronounced during the later stages of kernel development and paralleled the increased rate of transpiration associated with the severity of rust infection. The effect of transpiration on translocation seemed to occur about a week earlier in the case of stem rust, than in that of leaf rust, and continued until maturity.

Effect of Maturity on Phosphorus Content of Ash. An analysis of the data for the percentage of phosphorus in the ash showed that the severity of leaf or stem rust infection appeared to exert little material influence on the values. Accordingly, the data for variously treated samples within each variety, at corresponding maturity dates, were combined and their mean values recorded in Table V. The phosphorus content of the ash of the entire tillers of both varieties remained more or less constant as maturity progressed. However, as the plants matured, there was a relatively greater translocation of phosphorus than of the other ash constituents into the kernel. Thus, in Thatcher, the percentage of phosphorus in the kernel ash increased from 17.7% at 7 days after blossoming to 27.3% at full maturity; the percentage of phosphorus in the ash of the chaff (glumes and rachides) fell from 4.1% to 1.9%; while in the straw (stems and leaves), the phosphorus in the ash was reduced from 2.9% to 1.3% as the plant ripened. In Marquis, the preferential translocation of phosphorus into the kernel was less marked than in Thatcher, increasing from 21.1% to only 24.8% during the maturation period.

Precision of the Colorimetric Method for Total Phosphorus. The precision of the colorimetric method for phosphorus is shown by the data presented in Table VI. To determine whether the experimental errors were influenced by the magnitude of the phosphorus values, the samples were classified according to phosphorus content into three groups. Experimental errors were calculated from the duplicate determinations for the samples within each group and for the entire series. The absolute error did not increase proportionally with phos-

TABLE V

PHOSPHORUS CONTENT OF THE ASH OF VARIOUS FRACTIONS OF THE WHEAT PLANT AT SUCCESSIVE STAGES OF KERNEL DEVELOPMENT

Number of days after blossoming	Successive stages of kernel development	Percentage of phosphorus in ash of wheat plants			
		Grain	Chaff	Straw	Entire aerial tiller
		%	%	%	%
		Thatcher			
7	Pre-milk	17.7	4.1	2.9	3.5
10	Early milk	21.5	3.2	2.7	3.5
14	Late milk	22.5	2.4	2.4	3.6
17	Soft dough	24.9	2.1	2.2	3.9
21	Medium dough	26.1	1.8	1.7	3.9
24	Hard dough	26.6	1.8	1.4	3.8
28	Semiripe	26.8	1.8	1.2	3.7
31	Dead ripe	27.3	1.9	1.3	3.8
		Marquis			
7	Pre-milk	21.1	4.8	3.1	3.9
10	Early milk	22.5	4.1	2.8	3.9
14	Late milk	23.2	3.1	2.6	4.3
17	Soft dough	24.2	2.9	2.0	4.1
21	Medium dough	24.2	2.8	1.7	3.8
24	Hard dough	25.3	2.4	1.4	4.1
29	Near ripe	24.8	1.7	1.4	4.2

phorus concentration, although it is highest for the class representing samples which contained the highest percentage of phosphorus. The coefficient of variability decreased rather sharply with the increase in the mean values of the samples. The percentage errors show the precision of the method used in this study to be quite satisfactory.

TABLE VI

ERRORS OF THE COLORIMETRIC METHOD FOR DETERMINATION OF PHOSPHORUS

Class	Number of samples	Phosphorus (P)—dry matter basis			Coefficient of variability
		Class range	Class mean	Standard error per single determination	
		%	%	%	%
1	54	0.00–0.21	0.16	0.018	11.4
2	86	0.22–0.43	0.27	0.016	6.0
3	61	0.44–0.62	0.55	0.027	4.8
All	201	0.00–0.62	0.33	0.021	6.3

Summary

Total phosphorus determinations were made on three plant fractions grain (kernels), chaff (glumes and rachides), and straw (stems and leaves) prepared from tillers harvested during kernel filling from plots of Thatcher and Marquis wheats variously infected with leaf and stem rust, respectively. The leaf and stem rust epidemics were induced by artificial inoculations and the intensity of natural rust infection was controlled by periodic sulfur dustings. Average kernel weights and moisture contents of entire tillers were determined as indices of progressive maturity.

Average kernel weight was adversely affected by severe rust infection, particularly stem rust. Transpiration rate was accelerated to a greater extent by stem rust than by leaf rust, even though the average maximum stem rust infection was 48.6%, as compared with an average maximum of 89.5% leaf rust infection.

Percentage of phosphorus in the dry matter of the kernels remained more or less constant with progressive maturity. In the chaff (glumes and rachides) the percentage of phosphorus tended to decrease appreciably as the plants approached maturity, whereas in the straw (stems and leaves) a very consistent and most pronounced decrease occurred.

Total phosphorus in the entire tiller remained relatively constant throughout maturation, but the phosphorus was translocated from the straw and chaff into the developing kernel. The translocation of phosphorus exceeded that of the other ash constituents.

Severely rusted plants contained a somewhat higher concentration of phosphorus in the grain than the more lightly infected plants during the early stages of kernel development, but the differences were reduced as the plants approached maturity. Intensity of rust infection was without apparent influence on the percentage of phosphorus present in the chaff, but caused a definite increase in the concentration of phosphorus in the straw fraction in the final stages of maturation. The effects were similar in the case of leaf and stem rust.

Severe leaf rust infection, as that of stem rust, lowered the total phosphorus content of the entire tiller of the wheat plant and markedly interfered with its translocation into the kernels.

Literature Cited

Caldwell, R. M., Kraybill, H. R., Sullivan, J. T., and Compton, L. E.
1934 Effect of leaf rust (*Puccinia triticina*) on yield, physical characters, and composition of winter wheats. J. Agr. Res. 48: 1049–1071.

Geddes, W. F., and Levine, M. N.
1942 The distribution of thiamin in the wheat plant at successive stages of kernel development. Cereal Chem. 19: 547–552.

Greaney, F. J., Woodward, J. C., and Whiteside, A. G. O.
1941 The effect of stem rust on the yield, quality, chemical composition, and milling and baking properties of Marquis wheat. Sci. Agr. 22: 40 60.
Miller, E. C.
1939 A physiological study of the winter wheat plant at different stages of its development. Kansas State College of Agriculture and Applied Science. Tech. Bull. 47, 167 pp.

RAPID DETERMINATION OF BROMATE IN FLOUR

A. A. Towner and R. C. Koehn

General Mills, Inc., Wichita, Kansas, and Minneapolis, Minnesota

(Received for publication August 20, 1945)

Various techniques have been proposed from time to time for the estimation of bromate in flour, of which the most recent is that suggested by Geddes and Lehberg (1938). Although the method described by these authors is specific for bromate in the presence of other common oxidants, it apparently is slow and difficult, because they say, "A single determination requires approximately 8 hours and one analyst can conduct up to four determinations simultaneously." They also attempted to use a technique published by Kulman (1934), in which added bromate is separated by dispersing flour in carbon tetrachloride, but reported it to be unreliable.

Although we were not aware of Kulman's work at the time, we discovered several years ago that bromate was quite commonly present when carbon tetrachloride was used to separate added phosphates from certain export flours, and that a rough estimation of the amount of such bromate by the usual volumetric procedures was entirely feasible. Some time later we worked out the details of a satisfactory procedure based on carbon tetrachloride separation. Since we have obtained entirely satisfactory results using this technique, we are herewith offering the procedure in detail.

Reagents:

(1) Carbon tetrachloride.
(2) Starch indicator solution, 1%. Suspend 1 g soluble starch in a small volume of cold water, pour into boiling water, cool and dilute to 100 ml.
(3) Sulfuric acid, 10%.
(4) Potassium iodide solution, 50%.
(5) Sodium thiosulfate, 0.01 *N*.

Procedure:

Weigh 15 g flour into a 50 ml centrifuge tube containing about 20 ml of carbon tetrachloride. Disperse the flour thoroughly by means of a spatula or stirring rod but do not allow the flour to reach the bottom of the tube. Fill the tube to within one inch of the top with additional carbon tetrachloride and centrifuge for 2 minutes at a speed sufficient to effect a clean separation between flour and liquid.

Insert a stirring rod or spatula to the point of separation, and using a circular motion again disperse the flour. Centrifuge again for 2 minutes. Repeat dispersion with stirring rod, and centrifuge for 3 to 5 minutes or until the flour is densely packed and the carbon tetrachloride is clear.

Hold the tube in an inclined position and remove the flour by means of a small spoon or spatula. Carefully manipulate the tube until it is finally inverted and remove any flour still adhering by use of a small cotton swab, making sure that none of the solid residue at the bottom of the tube is lost. Evaporate residual carbon tetrachloride at room temperature.

Dissolve the residue in 5 to 6 ml water, add 2 ml 10% sulfuric acid, 1 ml potassium iodide solution, and 1 ml starch indicator solution and titrate with 0.01 *N* thiosulfate.

Potassium bromate, grams per cwt. = ml 0.01 *N* thiosulfate × 0.84.

In experimenting with additions of known amounts of bromate, we found that repeated resuspension of flour and centrifuging is necessary if reliable results are to be obtained. The more finely pulverized is the bromate employed, the more is it necessary to resort to multiple stage centrifuging. With these precautions, however, we have consistently obtained recoveries of 90 to 110% or better. The tabulation below gives results obtained in three different laboratories by this method on samples of flour to which known quantities of bromate had been added.

	Added bromate recovered		
Bromate added	Laboratory A	B	C
g per cwt.	%	%	%
0.75	100	—	—
1.0	99	—	—
2.0	99	—	—
2.3	102	100	94
4.5	99	98	97

This method cannot be applied to whole wheat flours which have been treated with bromate by dissolving the salt in water and applying to the whole grain. The procedure as given is not specific for bromate but will include any salt with density greater than that of carbon tetrachloride and which will oxidize an acidified iodide solution. Thus iodates, persulfates, etc., will be reported as bromate. It seems probable, however, that the technique suggested by Kolthoff and Hume (1943) could be applied to the residue and thus iodates and bromates could be quantitatively distinguished.

Literature Cited

Geddes, W. F., and Lehberg, F. H.
1938 The quantitative determination of bromine in bromated flours. Cereal Chem. 15: 49–58.
Kolthoff, I. M., and Hume, D. N.
1943 Determination of iodate in the presence of bromate and chlorate. Ind. Eng. Chem. (Anal. Ed.) 15: 174–175.
Kulman, J.
1934 A quantitative method for determining bromates in compounded flours. Chem. Listy. 28: 241–242. (Original not seen. Chem. Abstracts 29: 236. 1935.)

A RAPID METHOD FOR THE DETERMINATION OF BROMATE IN BROMATED FLOURS

A. Hoffer[1] and A. W. Alcock[2]

(Received for publication July 20, 1945)

For many years oxidizing agents such as potassium bromate, ammonium persulfate, nitrogen trichloride, and chlorine have been used as flour and dough improvers. Of these potassium bromate is one of the most commonly used and has, perhaps, been most widely studied. Probably close to 90% of all the spring wheat flour sold in Canada contains added bromate, the usual dosage being about 5 μg per g (5 ppm). A method, at once rapid and reasonably accurate, for the determination of a substance so frequently found in commercial flours is very much needed.

Geddes and Lehberg (1938) made a study of the subject, and of the methods they investigated found that the one described by Yates (1933) for the determination of bromine in blood best lent itself to the purpose in view. By an adaptation of this method, using special glass equipment designed by Binnington (1937), bromate in flour could be determined in the presence of other oxidizing agents such as periodates. The standard error of a single determination was 0.000097% (0.97 μg/g). Although four tests can be made simultaneously, a single determination by the method of Geddes and Lehberg takes about 8 hours, a fact which seriously limits the usefulness of the method for routine work.

Ford, Kent-Jones, Maiden, and Spalding (1940) also described a method for the determination of bromine in flour and provided an extensive review of the literature on the subject. Since the natural flours they examined varied in bromine content from 2.4 to 7.7 μg per g, the authors concluded that the additions of bromate usual in Great Britain, which increased the bromine content by an average of 3.6 μg per g, could not be detected by means of a bromine determination. In view of this, and because high bromine content may now be due to the use of methyl bromide as a fumigant, no reference will be made to more recent methods for determining bromine in flour.

A modification of the method outlined in *Cereal Laboratory Methods* (4th Ed., 1941) has long been used as a semi-quantitative test for bromate in flour. In brief, the method consists of dropping a mixed reagent of hydrochloric acid and potassium iodide on a smooth flour surface. The reagent is prepared just before use and is added drop by drop until the surface is uniformly wetted. Deep purple spots appear if bromate is present. With bromate of one uniform fineness, the number of spots per unit area is roughly proportional to the amount of the improver present in the flour. Unknowns can be compared with standards containing known dosages of the improver.

---

[1] Chemist, Purity Flour Mills Limited, Winnipeg, Manitoba.
[2] Chief Chemist, Purity Flour Mills Limited, Winnipeg, Manitoba.

The more precise method now to be described is based on the same reaction, but the flour is first extracted and the bromate in the extracts determined by measuring the intensity of the violet color produced upon the addition of acidified potassium iodide.

Experimental

Apparatus

(1) 250 ml Erlenmeyer flasks.
(2) 50 ml centrifuge tubes.
(3) Coleman Universal Spectrophotometer, Model 11, and optically matched round cuvettes of 1.7 cm diameter.

Reagents

(1) Potassium chloride solution, 25% in 2% acetic acid.
(2) Potassium permanganate solution, 5.0 mg/ml.
(3) 5% hydrochloric acid (c.p. free from chlorine).
(4) 1% potassium iodide solution.
(5) Mixed reagent. Equal parts by volume of 5% hydrochloric acid and 1% potassium iodide mixed immediately before use.
(6) Standard potassium bromate solutions, 2.5 μg/ml and 5.0 μg/ml. These solutions are prepared as required from a solution containing 100 μg/ml which is stable.
(7) 0.5% starch solution, prepared by the following method as outlined by Platner (1944): While stirring add approximately 30 ml of a 20% sodium hydroxide solution to a suspension of 2 g of soluble starch in 300 ml of water and allow to stand for 1 hour. Neutralize with concentrated hydrochloric acid using litmus as an indicator and then add 1 ml of glacial acetic acid. Dilute to 400 ml with water. This solution can be used for an indefinite period.

Method. Weigh 2.5 g of flour into a 250 ml Erlenmeyer flask if the sample contains less than 20 μg/g of bromate and 1.25 g if it contains between 20 and 50 μg of bromate per g. Add 49 ml of 25% potassium chloride solution in 2% acetic acid. Disperse the flour thoroughly and add 1 ml of permanganate solution, shaking the flask while doing so. If 1.25 g of flour is taken, use a solution containing 2.5 mg instead of 5 mg of permanganate per ml. Allow to stand at room temperature for 15 minutes with occasional shaking. Pour the mixed suspension into centrifuge tubes and centrifuge for 10 minutes or until clear. Pipette 10-ml aliquots into 4 test tubes. To tube (1) add 6 ml of distilled water; to tube (2) 1 ml of distilled water; to tube (3) 1 ml of standard bromate solution containing 2.5 μg/ml; and to tube (4) 1 ml of standard bromate solution containing 5 μg/ml. Prepare the mixed reagent and add 5 ml to each of tubes 2, 3, and 4. Immediately add 1 ml of starch solution to all four tubes, mix the contents of each, and exactly 10 minutes after the addition of the mixed reagent, read the optical density at the absorption peak.[3] Tube (1) is the blank.

The optical density is directly proportional to the concentration of bromate. The bromate concentration in the flour is determined from the increments in optical density due to additions of the standard bromate solutions. When 2.5 g of flour is used, the concentration is given by the formula:

$$\text{Concentration of bromate, } \mu g/g = \frac{2.5 \times A \times 50}{B \times 10 \times 2.5} = \frac{5A}{B}$$

where A is the optical density of the unknown in tube (2),
B is the average of the differences in optical density between tube (3) and tube (2), and between tube (4) and tube (3).

---

[3] In all the determinations reported in this paper the optical density was read at 575 mμ.

Effect of Potassium Chloride Concentration. The effect of concentration of the potassium chloride used for extraction was investigated over a range of 5 to 25% using a bleached and unbleached short patent and a bleached and unbleached first clear milled from Canadian hard spring wheat. Of each flour 2.5 g was extracted with 50 ml of potassium chloride solution of the indicated concentration in 2% acetic acid and to 10 ml of each clear extract 5 μg of potassium bromate was added. No permanganate was used. The mixed reagent and starch were then added and after 10 minutes the optical density readings were taken. The results in Table I show that increasing the concentration of the potassium chloride from 5 to 25% greatly increased the optical density. Because it gave the maximum readings, the 25% solution was adopted as the extractant. At all levels of potassium chloride concentration the bleached patent gave higher readings than the unbleached patent, and the unbleached patent higher readings than the clear. Before the method could be considered to possess any promise whatever, these discrepancies – apparently caused by variations in the quantity of bromate reduced [1] by the extracts of different flours while the test was being carried out had to be eliminated.

TABLE I

OPTICAL DENSITY PRODUCED BY 5 μG OF POTASSIUM BROMATE IN FLOUR EXTRACTS AS INFLUENCED BY CONCENTRATION OF POTASSIUM CHLORIDE

Flour			Optical density				
			Concentration of KCl				
Grade	Ash %	Bleach	5%	10%	15%	20%	25%
Patent	0.38	None	0.064	0.076	0.133	0.147	0.165
Patent	0.38	Agene and Alsop	0.123	0.170	0.210	0.225	0.240
Clear	0.82	None	0.002	0.004	0.008	0.010	0.012
Clear	0.82	Agene and Alsop	0.000	0.005	0.005	0.014	0.014
Mean			0.047	0.064	0.089	0.099	0.108

Effect of Permanganate. To destroy the substances responsible for the loss of bromate, various oxidizing reagents were tried and of these potassium permanganate was found to be most suitable. It oxidized the flour extracts to the extent that they no longer reduced bromate while the determinations were being carried out and was itself so quickly and completely reduced that none was left to liberate iodine when iodide (mixed reagent) was added. *The same results were obtained whether the permanganate was added to the flour suspension before or after the addition of the bromate.*

---

[1] Though we speak of the bromate being reduced, its disappearance can be just as well explained on the theory that it is adsorbed on some constituent of the flour extract. In a subsequent paper we hope to submit evidence in support of the latter explanation.

The same flours previously used were extracted as before except that the extractant now consisted of 49 ml of potassium chloride solution and 1 ml of a solution containing 2.5 mg of potassium permanganate. Varying concentrations of potassium chloride were again used and to a 10-ml aliquot of each clarified extract 5 μg of bromate was again added. Results of optical density measurements, taken 10 minutes after the addition of the mixed reagent, are given in Table II.

TABLE II

OPTICAL DENSITY PRODUCED BY 5 μG OF POTASSIUM BROMATE IN FLOUR EXTRACTS AS INFLUENCED BY THE CONCENTRATION OF POTASSIUM CHLORIDE, PERMANGANATE BEING ADDED TO THE EXTRACT

Flour		Optical density				
		Concentration of KCl				
Grade	Bleach	5%	10%	15%	20%	25%
Patent	None	0.114	0.175	0.190	0.220	0.235
Patent	Agene and Alsop	0.115	0.172	0.210	0.220	0.247
Clear	None	0.130	0.178	0.198	0.235	0.260
Clear	Agene and Alsop	0.120	0.158	0.200	0.230	0.255
Mean		0.120	0.171	0.200	0.226	0.249

A comparison of the results in Tables I and II shows that the addition of permanganate to the extractant had no effect on the intensity of the color developed in the bleached patent extracts, but caused pronounced increases in the readings for the unbleached patent and still greater increases with the clear. With all flours the more concentrated potassium chloride solutions again gave the higher optical densities.

The effect of permanganate may be seen more clearly from the calibration curves of optical density against bromate concentration shown in Figure 1. The plotted results were obtained as follows: To 2.5 g of the flour a mixture of 49 ml of 25% potassium chloride solution in 2% acetic acid and 1 ml of a solution containing 2.5 mg of potassium permanganate was added, followed by the specified amount of bromate as soon as the permanganate color had disappeared. The determination of optical density was then carried out according to the usual procedure. The experiment was repeated using 50 ml of potassium chloride solution without permanganate. An unbleached feed flour

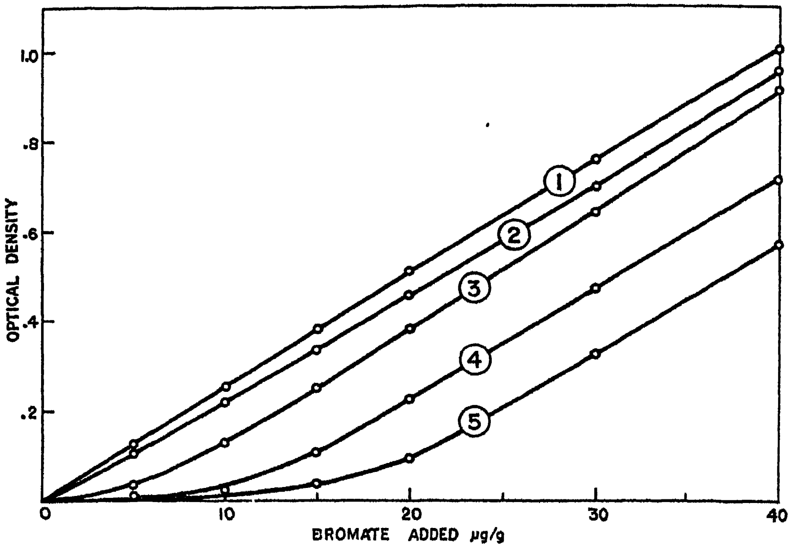


Fig. 1. Effect of increments of potassium bromate, with and without $KMnO_4$, on optical density. Curve (1) bleached and unbleached patent and clear flours with $KMnO_4$ and bleached patent without $KMnO_4$; (2) feed flour with $KMnO_4$, (3) unbleached patent, (4) bleached clear, (5) unbleached clear—without $KMnO_4$.

having an ash content of 1.40% was included in the permanganate series.

With permanganate in the extractant, the bleached and unbleached patent and clear, as well as the bleached patent without permanganate, gave five sets of results which were in such close agreement that they were taken to produce a single curve. Apparently no loss of bromate occurred while these tests were being made. Without permanganate the curve for the unbleached patent was logarithmic at lower bromate concentrations and a straight line at higher concentrations, while the curves for the clear flours became straight lines only at the highest concentrations of bromate. These three curves are interpreted to mean that, except in the straight line portions, bromate losses were taking place and that these losses were related to the bromate concentration and the degree of oxidation (grade and bleach) of the flour. By extrapolating the straight line portions of the curves to zero optical density, the maximum loss for each flour can be estimated as follows: Unbleached patent 4 to 5 µg per g of flour, bleached clear 11 µg, and unbleached clear 16 µg.

Feed flour with permanganate gave a straight line curve which, however, upon extrapolation, did not pass through the origin, the indicated loss of bromate being 1 µg per g of flour. It was a very low-grade product and was used, not as an example of a bromated flour of commerce but in order to include flours at both extremes of the grade scale.

The effect on the optical density of using different quantities of permanganate was studied with results shown in the curves which make up part of Figure 2.

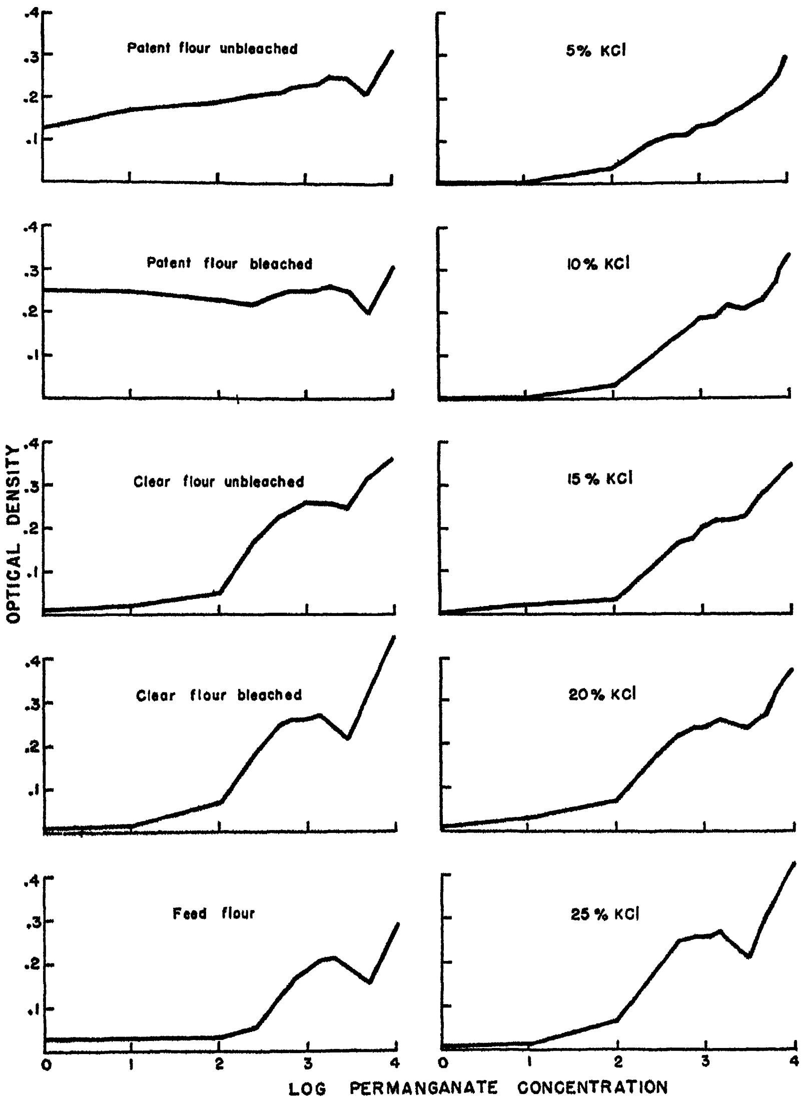


Fig. 2. Effect on optical density of varying permanganate concentration from 1 μg to 10,000 μg per g of flour. (Left) Using different grades of flour. (Right) Using different concentrations of KCl with the same flour (bleached clear).

Each flour was suspended in the 25% potassium chloride solution, permanganate was added, and this was followed by 10 µg of bromate per g of flour, after the permanganate color had disappeared. The regular procedure was then followed. The curves differ rather widely, but they show that for each flour there is a range of permanganate additions which produce almost constant optical density readings. These ranges were as follows:

Flour	Range of $KMnO_4$ additions producing uniform optical density	Mean optical density within $KMnO_4$ range
Patent unbleached	0.5 to 3 mg/g of flour	0.232
Patent bleached	0 to 3 mg/g of flour	0.246
Clear unbleached	0.5 to 3 mg/g of flour	0.251
Clear bleached	0.5 to 2 mg/g of flour	0.256
Feed flour	1.0 to 3 mg/g of flour	0.200

From Figure 1, it was concluded that a small loss of bromate occurred when 1 mg of permanganate was used per g of feed flour. Since a larger quantity of permanganate apparently failed to reduce this loss, bromate cannot be accurately determined in such very low-grade flour by the proposed method. For the determination of bromate in the short patent and clear flours used in these experiments, the addition of from 0.5 to 2 mg of permanganate per g of flour gave satisfactory results. In our own application of the method we have used 2 mg.

Table II showed that, using 1 mg of permanganate per g of flour, higher optical densities were obtained with more concentrated potassium chloride solutions. It seemed of interest to determine whether this was due to a salt effect on the intensity of the color or to losses of bromate at lower potassium chloride concentrations. In the latter case an adjustment of the quantity of permanganate might be expected to prevent these losses and thus bring into line the optical densities obtained with different concentrations of potassium chloride. The procedure used in the preceding experiment was adopted, but instead of using different flours, the same flour (bleached clear) was suspended in potassium chloride solutions of different strength made up as always in 2% acetic acid. The combined effect of variations in permanganate and potassium chloride concentrations is shown by curves reproduced in Figure 2.

The results indicated that the more concentrated the potassium chloride solution the less reducing material it extracts. The plateaus in the curves for 20 and 25% potassium chloride are wider and occur at lower permanganate concentrations than those in the curves for the 10 and 15% solutions. The 5% curve showed no plateau, the optical density increasing progressively with increasing amounts of permanganate. Not all of these features are well shown by the separate log curves, but the essential data are as follows:

KCl concentration	Range of $KMnO_4$ additions producing uniform optical density	Mean optical density within $KMnO_4$ range
25%	0.5 to 2 mg/g of flour	0.256
20%	0.75 to 3 mg/g of flour	0.246
15%	1.5 to 3 mg/g of flour	0.223
10%	2 to 5 mg/g of flour	0.220
5%	—	—

In strong potassium chloride extracts little bromate-reducing material is present and this is satisfied by small additions of permanganate. In the 25% extract 0.5 mg of permanganate was sufficient, but amounts up to 2 mg did not materially alter the readings. The extra permanganate was evidently reduced to produce compounds which did not react with iodide. Oxidation with still larger amounts of permanganate, however, led to the formation of products capable of liberating iodine from iodide. (No explanation can be suggested for the dips in the curves shown in Figure 2.)

When much reducing material is present in solution, as is the case with the 5% potassium chloride extract, relatively large quantities of permanganate must be added to prevent loss of bromate, if, indeed, it is completely prevented. Probably before this stage is reached, oxidation products that react with iodide have already been formed, with the result that there is no plateau in the curve.

The test thus depends on the fact that 25% potassium chloride in 2% acetic acid extracts bromate without, at the same time, taking up relatively large amounts of strongly reducing substances.

The preceding experiment shows that the low optical density results in Table II were due to the use of insufficient permanganate or, in the 5% potassium chloride extract, to the presence of interfering material. That it was not due to a salt effect on the color is confirmed by the results of the next experiment.

Extracts of bleached and unbleached clear were made with different concentrations of potassium chloride, and to one aliquot of each extract, bromate equivalent to 10 µg per g of flour was added and to another the equivalent of 20 µg per g. The differences in optical density readings between the pairs of aliquots are reported in Table III.

Variations in the potassium chloride concentration from 10 to 25% had little if any effect on the optical density.

Effect of Time and Temperature of Extraction. The results were unaffected by varying the time of extraction from 15 to 60 minutes or by varying the temperature of a 15-minute extraction from 0° to 50°C. Bromate losses during extraction in the absence of permanganate

TABLE III

INCREASE IN OPTICAL DENSITY OF EXTRACTS CONTAINING 20 μG/G OF POTASSIUM BROMATE OVER EXTRACTS CONTAINING 10 μG/G OF BROMATE AS INFLUENCED BY CONCENTRATION OF POTASSIUM CHLORIDE

Flour and treatment	Optical density				
	Concentration of KCl				
	5%	10%	15%	20%	25%
Bleached clear	0.210	0.242	0.245	0.250	0.245
Unbleached clear	0.220	0.242	0.247	0.250	0.250

increased at 70°C, and when the temperature was raised to 100° all the bromate was reduced.

Stability of Color. When the optical density of a blank, prepared by adding mixed reagent to an unbromated flour extract in 25% potassium chloride, was deducted from the optical density of the same extract containing bromate, it was found that the color intensity due to the bromate increased for 6 or 7 minutes after the addition of iodide and then remained stationary. The optical densities of the bromated extract and the blank continued, however, to show an upward drift. In the interval from 10 to 20 minutes after the mixed reagent was added, the mean increase in 16 experiments was 0.015, equivalent to 0.6 μg per g of bromate. This increase took place whether permanganate was added or not and was due to the slow liberation of iodine by other reagents, by substances other than bromate in the flour extract, or by dissolved oxygen. Because of this change in color with time, readings must be made exactly 10 minutes after the mixed reagent is added.

Characteristics of the Starch-Iodine Color. In our analyses of commercial flours, the starch-iodine solutions showed a maximum light absorption at a wave length of approximately 575 mμ and we have used that wave length consistently. Soluble starch is added to the extracts in order to ensure an excess, especially when dealing with heavily bromated flour. When iodine reacted with the soluble starch alone, the maximum light absorption was found to be at 600 mμ. In spite of the addition of soluble starch, the absorption characteristics are always largely determined by the nature of the reacting carbohydrates extracted from the flour. Fortunately, small variations in the absorption maximum can be safely ignored since the absorption peak is broadly rounded and because an internal standard is used.

The potassium chloride extract of a 15-year-old flour (with the usual amount of added soluble starch) showed a maximum absorption at 420 mμ, but some factor other than age alone must have been responsible for this low reading, for on testing a sample 39 years old, its peak was found to be at 550 mμ. It is not known to what extent the absorption characteristics of the starch-iodine solution are influenced by the type and grade of wheat from which flours are produced or by other factors such as flour storage conditions. Certain low-grade flour streams give solutions having a maximum absorption at lower wave lengths, i.e., they are more reddish in color than usual. In case of doubt the absorption characteristics should be investigated, as may be quickly and easily done by means of a spectrophotometer.

Interference by Other Improvers. Of the following oxidizing agents which were added to flour at a concentration of 100 μg per g, potassium persulfate, ammonium persulfate, potassium iodate, potassium periodate, potassium chlorate, and potassium perchlorate, only the iodate and periodate reacted with the mixed reagent. Consequently, bromate cannot be estimated by the rapid method in the presence of either of these two salts. Ordinary dosages of nitrogen peroxide, nitrogen trichloride, and chlorine do not interfere with the test. Heavily chlorinated flours may react with the mixed reagent, but such flours are seldom, if ever, treated with bromate.

Precision of the Rapid Method. Twenty bromated flours were analyzed in duplicate. The mean of all the determinations was 13.17 μg per g and the mean difference between duplicates, which were run on different days, 1.01 μg per g. The standard error of a single determination was 0.97 μg per g.

To find what part of the error was associated with the steps involved in the development and measurement of the color, two aliquots of each of 16 extracts of bromated flours were taken for the measurement of optical density. The standard error was found to be 0.41 μg per g, or nearly half the total error.

Summary

Potassium bromate in flour may be quickly determined by extracting with 25% potassium chloride in 2% acetic acid, allowing the extracted bromate to react with acid potassium iodide, and measuring the optical density of the amylose-iodine solution at the maximum light absorption.

The method depends upon the fact that concentrated potassium chloride solution extracts only small quantities of interfering substances. Sufficient permanganate is added to the extractant to destroy these substances without the formation of oxidation products which react with iodide.

Below 50°C the temperature and time of extraction are not critical factors.

Bromates in commercial flours have been determined by measuring optical densities at a fixed wave length of 575 mμ, but wider experience may show the necessity for varying this wave length according to the absorption characteristics of the starch-iodine solution.

Spring wheat flours ranging in grade from short patents to first clears have been analyzed by the method. It cannot be applied to very low-grade flours since they yield extracts which react with bromate while the test is in progress.

Bromate cannot be determined by the proposed method in the presence of iodate or periodate. Other improvers, when used in the dosages commonly applied to bread flour, do not interfere.

The standard error of a single determination is 0.97 μg per g of which approximately half is due to errors in sampling and extraction.

Literature Cited

Binnington, D. S.
1937 An improved reaction microapparatus. Ind. Eng. Chem. (Anal. Ed.) 9: 353.
Ford, W. P., Kent-Jones, D. W., Maiden, A. M., and Spalding, R. C.
1940 The micro-determination of bromine in foodstuffs. J. Soc. Chem. Ind. 59: 177–180.
Geddes, W. F., and Lehberg, F. H.
1938 The quantitative determination of bromine in bromated flours. Cereal Chem. 15: 49–58.
Platner, W. S.
1944 Stable starch solution for dissolved oxygen determinations. Ind. Eng. Chem. (Anal. Ed.) 16: 369.
Yates, E. D.
1933 The determination of bromine in blood. Biochem. J. 27: 1763–1769.

THE DISTRIBUTION OF THIAMINE IN WHEAT SEEDLINGS AT DIFFERENT STAGES OF GERMINATION IN THE DARK[1]

A. HOFFER,[2] A. W. ALCOCK,[3] and W. F. GEDDES[4]

(Received for publication June 4, 1945)

Inquiries arising from the efforts of nutritionists to increase the vitamin content of the diet have led to a suggestion that the use of germinated cereals should be encouraged (Nutrition Reviews, 1943). Burkholder and McVeigh (1942) reported significant increases in riboflavin, niacin, biotin, and pyridoxine during germination of cereals but found no change in total thiamine, although the concentration of this vitamin in green seedling leaves was high compared with that in the original seed. A later summary presented by Burkholder (1943) pointed to the conclusion that a slight increase in thiamine, accompanied by very marked increases in riboflavin and niacin, occurred when seeds were germinated in a greenhouse for five or six days. On the other hand, McVeigh (1944) found no significant change in the thiamine content of oat seedlings which were allowed to grow in the dark for five days. The leaf showed absolute increases; the coleoptile little or no change. Davis, Laufer, and Saletan (1943) reported that malted barley and wheat were much higher in riboflavin, a little higher in niacin and pantothenic acid, and a little lower in thiamine than the unmalted samples. From these reports it appears that when cereals are allowed to germinate in the light, small increases take place in the thiamine content of the plant as a whole, but in the dark there is no increase in total thiamine, though some transfer from seed to leaf occurs. The purpose of the present study was to investigate the content and distribution of thiamine in the organs of the wheat seedling at successive stages of germination in the dark.

---

[1] Paper No. 2251, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Chemist, Purity Flour Mills Limited, Winnipeg, Manitoba.

[3] Chief Chemist, Purity Flour Mills Limited, Winnipeg, Manitoba.

[4] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

Experimental

A sample of Marquis wheat was steeped to 44% moisture at 10°C (50°F) and transferred to a dark germination chamber, described by Meredith, Hlynka, and Sallans (1944), that was maintained at 11.7°C (53°F) and 100% relative humidity.

Samples were removed at intervals over an 18-day period and a selected number of seedlings immediately dissected into kernel and sprout sections. The kernel was divided transversely into approximately equal brush and germ portions. At the end of the fourth day the sprout was large enough to divide into upper and lower fractions. The upper fraction comprised the coleoptile, stem, and any foliage leaves that had formed; the lower fraction consisted of the scutellar node and the rootlets. To locate the thiamine in the sprout in greater detail, the lower fraction of the samples germinated for 18 days was further dissected into two portions, one consisting of the root system and the other the scutellar node. The various fractions were air-dried, weighed, and analyzed for thiamine by the regular thiochrome method outlined in *Cereal Laboratory Methods* (4th ed., 1941), except that the sodium hydroxide and potassium ferricyanide solutions were combined before use. Sample and extractant were thoroughly mixed by means of a Waring Blendor. The determinations were made both with and without takadiastase and all the results are reported on an air-dry basis (approximately 10% moisture).

Results and Discussion

The distribution of the air-dry weight of the seedling between the two kernel fractions and the sprout, together with the values (using diastase) for thiamine concentration, total thiamine per 100 seedlings, and the distribution of the total thiamine of the seedling in the three fractions at different periods of growth are shown graphically in Figure 1. The corresponding data for the upper and lower fractions of the sprout in comparison with the entire sprout are represented in Figure 2.

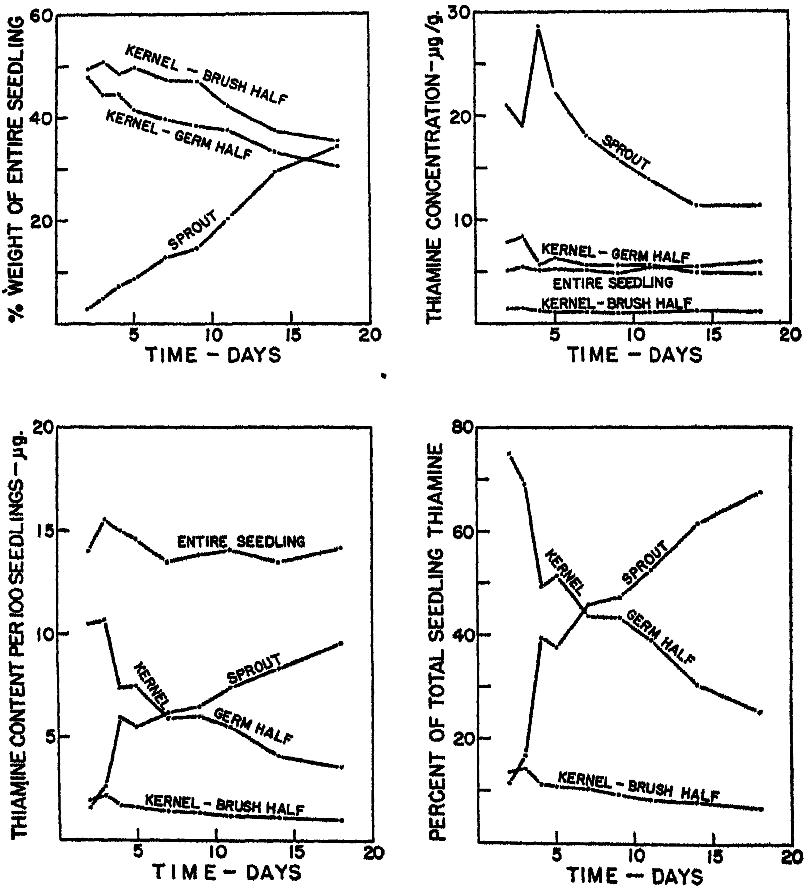


Fig. 1. Weight distribution and the concentration, content, and distribution of thiamine in different fractions of the wheat seedling at successive stages of germination in the dark.

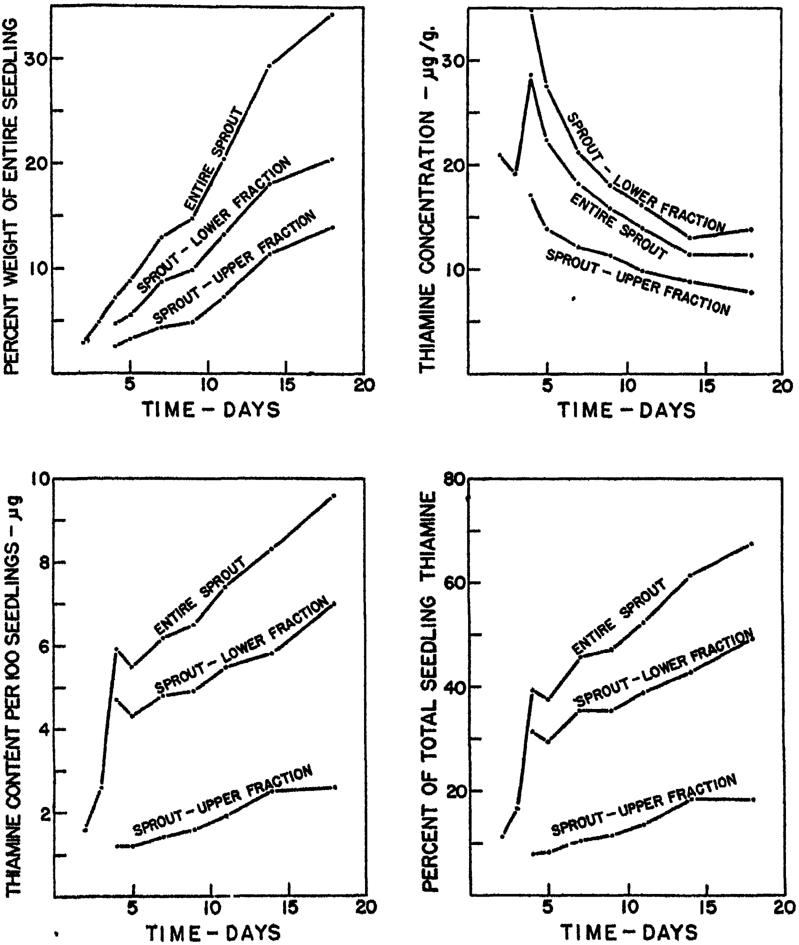


Fig. 2. Weight distribution and the concentration, content, and distribution of thiamine in the entire wheat sprout and its upper and lower fractions at successive stages of germination in the dark. The upper fraction of the sprout comprised the coleoptile, stem, and any foliage leaves that had formed, the lower fraction consisted of the rootlets and scutellar node.

The air-dry weight of the sprout relative to that of the entire seedling increased regularly from approximately 3% at 2 days germination to about 34% at 18 days germination.

The thiamine concentrations in the brush and germ halves of the kernel at two days germination were 1.4 and 7.9 µg/g air-dry weight respectively as contrasted with 20.9 µg/g in the sprout. After an apparent slight decrease between the second and the fourth day, the thiamine concentration of the brush half of the kernel remained relatively constant at approximately 1.1 µg/g during the remainder of the 18-day period. In the germ half, however, there was a decrease to

4.8 μg/g as germination progressed—a decrease that probably occurred at a more uniform rate than was indicated by values influenced by errors in dissection and assay. In the sprout, the thiamine concentration fell rapidly (after an apparent increase at 4 days) to 11.3 μg/g at 18 days. In the seedling as a whole the concentration showed little change except for a small increase during the later stages of germination when the air-dry weight of the seedling slightly decreased. The lower fraction (rootlets and scutellar node) of the sprout was much richer in thiamine than the upper fraction (coleoptile, stem, and foliage leaves). At 4 days germination the thiamine concentration in the lower fraction was 34.9 μg/g as compared with 17.1 μg/g in the upper fraction; the corresponding values at 18 days germination were 13.8 and 7.7 μg/g (Figure 2). When the lower fraction was further subdivided on the eighteenth day into rootlets and nodal tissue, it was found that the thiamine concentration in the scutellar node was considerably higher than in the rootlets (Table I).

TABLE I

Distribution of Thiamine, Including Cocarboxylase, in Wheat Seedlings after 18 Days Germination in the Dark

Fraction of plant	Weight per 100 plants[1]	Distribution of weight	Thiamine[1] concentration	Thiamine content per 100 seedlings	Distribution of thiamine
	g	%	μg/g	μg	%
Sprout					
Upper fraction[2]	0.344	13.9	7.7	2.6	18.3
Scutellar node	0.268	10.8	17.5	4.7	33.1
Rootlets	0.238	9.6	9.6	2.3	16.2
Kernel					
Germ half	0.749	30.3	4.8	3.6	25.4
Brush half	0.871	35.4	1.1	1.0	7.0
Entire seedling	2.470	100.0	5.8	14.2	100.0

[1] Air-dry basis (approximately 10% moisture).
[2] Coleoptile, stem, and foliage leaves.

Because of changes in the weights of the various fractions, the concentration data fail to indicate the changes in the thiamine content of the seedling and its fractions as germination progresses. These can be seen by computing the total thiamine values per hundred seedlings or seedling fractions. No significant change occurred in the quantity of thiamine present in the entire seedling, but the sprout increased in thiamine at the apparent expense of the brush and germ halves of the kernel, particularly the latter. From the second to the eighteenth day the percentage of the total thiamine of the seedling present in the sprout increased from 11% to 68%, while in the germ end of the kernel it decreased from 75% to 25% and in the brush end from 14% to 7%. The decrease in the total thiamine present in the brush end began at the fourth day when the germ end had already lost about one-third of the thiamine it had contained on the second day. The total thiamine content of the upper and lower fractions of the sprout increased at approximately the same relative rate (Figure 2). After 18 days roughly half the thiamine in the sprout was in the nodal tissue, the remainder being about equally divided between the rootlets and the coleoptile-stem-leaf fraction (Table I). The increase in the total thiamine content of the sprout took place relatively faster than the increase in air-dry weight, especially during the early stages of growth. Thus on the fifth day the sprout contained about 9% of the air-dry weight and 38% of the thiamine in the entire seedling, and at the eighteenth day, 34% of the weight and 68% of the thiamine.

The increase in the proportion of the total thiamine of the seedling which is found in the sprout as germination proceeds does not necessarily prove that thiamine is translocated from the kernel (particularly the germ half) during germination. The data can also be explained by assuming that the vitamin is utilized and destroyed in the kernel, whereas it is synthesized in the sprout. However, since the total thiamine in the entire seedling remains virtually constant throughout the whole period of germination, this interpretation implies an exact balance between the rate of destruction and of synthesis. It therefore seems more logical to interpret the results as indicative of the translocation of thiamine from the kernel to the sprout where it is required for the active respiratory processes occurring there. McVeigh (1944), however, in a similar study of the distribution of B vitamins in oat seedlings considers that vitamin synthesis occurs in the sprout (particularly the leaves). She states that as the seedling grows, the vitamins are constantly being used so that "the assays do not indicate the total amounts of vitamins produced within the seedling but rather the excess of production over the amounts used." In her studies with oat seedlings grown in the dark, she found significant increases in total riboflavin and niacin but little or no change in total thiamine.

Results of thiamine determinations without the use of takadiastase are reported in Tables II and III as free thiamine; the differences between these values and those obtained when enzyme was used are reported as bound thiamine. Since they are influenced by errors of both determinations, the bound thiamine values are not very reliable, but they suffice to show that germinating wheat contains very little cocarboxylase and that most of it is in the sprout. From the results reported by Hoffer, Alcock, and Geddes (1943) with respect to the cocarboxylase content of commercial wheat germ and other mill products, it appears that little, if any, synthesis of this substance occurs during germination, or that destruction balances synthesis.

TABLE II

FREE AND BOUND THIAMINE CONCENTRATION OF FRACTIONS OF WHEAT SEEDLINGS AT SUCCESSIVE STAGES OF GERMINATION IN THE DARK

Germination period	Free thiamine concentration[1]					Bound thiamine concentration[1]				
	Kernel		Sprout		Entire seedling	Kernel		Sprout		Entire seedling
	Brush half	Germ half	Lower fraction[2]	Upper fraction[3]		Brush half	Germ half	Lower fraction[2]	Upper fraction[3]	
Days	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
4	1.0	5.5	30.2	12.9	4.8	0.2	0.2	4.7	4.2	0.3
5	1.0	5.8	23.4	11.8	4.6	0.1	0.5	4.1	2.0	0.6
7	1.0	5.2	17.6	11.3	4.5	0.1	0.4	3.5	0.8	0.6
9	1.1	5.7	16.7	11.6	4.9	0.0	0.0	1.3	0.0	0.0
11	1.0	5.5	13.3	9.9	5.0	0.1	0.1	2.8	0.0	0.4
14	1.1	4.7	12.4	7.7	5.1	0.1	0.2	0.6	1.1	0.3
18	1.1	4.9	12.7	6.6	5.4	0.0	0.0	1.1	1.1	0.4

[1] Air-dry basis (approximately 10% moisture)
[2] Rootlets and scutellar node
[3] Coleoptile, stem, and any foliage leaves that have formed

TABLE III

DISTRIBUTION OF FREE AND BOUND THIAMINE IN WHEAT SEEDLINGS AFTER 18 DAYS OF GERMINATION IN THE DARK

Fraction of plant	Thiamine[1] concentration		Thiamine content per 100 plants		Distribution of thiamine	
	Free	Bound	Free	Bound	Free	Bound
	μg/g	μg/g	μg	μg	%	%
Sprout						
Upper fraction[2]	6.6	1.1	2.3	0.3	17.3	33.3
Scutellar node	16.9	0.6	4.5	0.2	33.8	22.2
Rootlets	8.1	1.5	1.9	0.4	14.3	44.5
Kernel						
Germ half	4.9	0.0	3.6	0.0	27.1	0.0
Brush half	1.1	0.0	1.0	0.0	7.5	0.0
Entire seedling	5.4	0.4	13.3	0.9	100.0	100.0

[1] Air-dry basis (approximately 10% moisture).
[2] Coleoptile, stem, and any foliage leaves that have formed.

The results obtained in this study, like those reported by others, show that the thiamine content of wheat cannot be increased by germination in the dark. Indeed, if the sprouts are removed during subsequent processing, as is the case when barley is malted, appreciable losses of thiamine will occur. But if the total thiamine content of germinating wheat does not increase, neither does it decrease. Either thiamine was not destroyed as a result of its participation in the physiological activities associated with germination and seedling growth, or the rates of destruction and synthesis were equal.

A single experiment showed that when the 18-day seedlings were exposed to the light, synthesis of thiamine occurred very rapidly. The synthesized thiamine was found distributed over the kernel, and the various fractions of the sprout.

Summary

Total thiamine content of wheat seedlings grown in the dark was essentially constant over an 18-day germination period at 11.7°C. From the second to the eighteenth day, the percentage of the total thiamine present in the sprout increased from 11% to 68%, while in the germ end of the kernel it decreased from 75% to 25% and in the brush end from 14% to 7%. These results indicate that thiamine is translocated from the kernel to the developing sprout. Preferential translocation of thiamine relative to total air-dry material was most pronounced during the early stages of growth.

Thiamine concentrations of 1.4, 7.9, and 20.9 μg/g air-dry material respectively in the brush half, germ half, and sprout at 2 days germination fell to 1.1, 4.8, and 11.3 μg/g respectively at 18 days germination.

Thiamine concentration in the fraction of the sprout comprising the rootlets and scutellar node was approximately twice that of the remaining structures (coleoptile, stem, and leaves). Further dissection of the sprout after 18 days germination showed the scutellar node to have a much higher thiamine concentration than any other part of the seedling.

Acknowledgment

The authors gratefully acknowledge the aid received from K. Hlynka, Experimental Miller, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba, who germinated the samples for this study.

Literature Cited

Burkholder, P. R.
1943 Vitamins in dehydrated seeds and sprouts. Science 97: 562–564
— and McVeigh, I.
1942 Increase of B vitamins in germinating seeds. Proc. Natl. Acad. Sci. U. S. 28: 440–446.
Davis, C. F., Laufer, S., and Saletan, L.
1943 A study of some of the vitamin B complex factors in malted and unmalted barley and wheat of the 1941 crop. Cereal Chem. 20: 109–113.
McVeigh, I.
1944 Occurrence and distribution of thiamine, riboflavin, and niacin in Avena seedlings. Bull. Torrey Botan. Club 71: 438–444.
Meredith, W. O. S., Hlynka, K., and Sallans, H. R.
1944 Laboratory Malting IV. A germination chamber for routine malting tests. Cereal Chem. 21: 251–258.
Nutrition Reviews
1943 B vitamins in sprouted cereal grains. 1: 356.

FURTHER STUDIES ON THE MECHANISM OF THE ACTION OF OXIDATION AND REDUCTION ON FLOUR

MARJORIE HOWE

Russell-Miller Milling Company Research Laboratory, Minneapolis, Minnesota

(Received for publication June 22, 1945)

The mechanism of the action of oxidation and reduction on flour has long been the subject of extensive research and considerable controversy. The theory that sulfhydryl compounds affect dough by means of activation of naturally occurring proteolytic enzymes in the flour has been forwarded by Jørgensen (1935, 1938) and by Balls and Hale (1936, 1938). On the other hand, this point of view has been opposed by groups who interpret the action of sulfhydryl as affecting the gluten structure. The investigation of Baker, Parker, and Mize (1942, 1943) on the role of pentosans in affecting dough quality offers a possible mechanism of action of oxidizing and reducing agents that excludes both the proteolytic theory and the view of direct chemical protein alteration. Actually there is no conclusive proof which theory is correct since all of the work reported gives data which are at best only suggestive and the interpretation varies widely.

The foundation on which the proteolytic theory has been developed began with the conclusion of Jørgensen (1935) that flour contains "powerful but latent" proteolytic enzymes of the papain type. Jørgensen (1938) supported this point of view by experiments demonstrating a decrease in the liberation of water-soluble nitrogen in flour extracts containing added bromate. Balls and Hale (1938) used their demonstration of the papain nature of the proteolytic enzyme in wheat bran as a prop to the theory; and in a later paper (1940) they found a sulfhydryl-containing substance in the petroleum ether extract of flour which they stated might serve as a natural protease activator that could, in this capacity, modify the gluten. Later, Elion (1943) attempted to lend credence to the proteolytic theory by experiments showing that addition of glutathione or a papain preparation to dough had a softening effect, while dough made from heated flour was softened only by the papain preparation. Since the latter must have contained sulfhydryl compounds, there is no conclusive evidence that the protease in the preparation played a role. Even in the case of the heated flour, the differences observed may have been due to the quantitative difference in sulfhydryl applied as glutathione and in the papain preparation. There can be no doubt concerning the fact that addition of sulfhydryl compounds softens dough, but this fact alone constitutes no proof that the effect is due to proteolysis rather than some other mechanism.

On the basis of a variety of hypotheses, other workers such as Bungenberg de Jong (1938), Ford and Maiden (1938), Read and Haas (1939), and Swanson and Dines (1939) opposed the proteolytic theory. Opposition was also presented by Sullivan, Howe, Schmalz, and Astleford (1940) whose work indicated that changes in the sulfur linkages in the gluten proteins could be responsible for the effects of oxidizing and reducing substances on dough. This point of view was strengthened by the work of Olcott, Sapirstein, and Blish (1943) indicating that the primary effect of reducing agents on gluten and dough is a chemical one upon the proteins and only secondarily that of enzyme activation. Sandstedt and Fortmann (1943) state that the action of reasonable quantities of reducing agents is reversible by oxidation, but the action of papain is not. Because the action of naturally occurring reducing agents in flour may be reversed by subsequent oxidation, these authors give this as evidence that the reduced character of doughs and bread made from unoxidized flour is not due to proteolysis.

The reversibility of oxidation and reduction of the sulfhydryl groups as they exist in gluten, in glutathione, or as a constituent part of any proteolytic enzyme, needs to be interpreted with a great deal of caution.

In their work on the effects of cysteine, glutathione, and papain on gluten demonstrated by gluten recovery and mixogram patterns, Swanson and Andrews (1945) found that, unlike reducing agents, papain affected the amount of gluten recovered and this amount was greatly decreased by increasing the amount of papain added or by lengthening the rest periods on the dough mixer. These authors could detect no activation of the latent proteases present in flour by the action of yeast-water, cysteine, or glutathione.

The purpose of this paper is to test the proteolytic theory by studying the effects of flour protease inhibitors on farinograph curves in order to determine whether the effects observed are compatible with those that would be predicted on the basis of the theory. Sodium fluoride and hexylresorcinol were employed for this purpose since Howe and Glick (1945) showed them to be potent inhibitors of wheat protease.

Experimental

An untreated patent flour milled from a Northwest spring wheat was chosen for this study. A water extract of wheat germ, prepared and measured for proteolytic activity as described previously by Howe and Glick (1945), was used as a rich source of protease. Farinograph curves were obtained in the usual fashion, with the water replaced by each of the following: a *M*/7 acetate buffer of pH 5.0, an acetate buffer-germ extract mixture (1 : 1), and the buffer-extract mixture containing 2.5 mg hexylresorcinol or 4.0 mg sodium fluoride per ml of germ extract.

In addition, the inhibitors were used in the buffer solution without germ extract; and a germ extract was also employed without buffer in which the enzyme was inactivated, as shown by titration, by heating at 80°C for 35 minutes. Howe and Glick (1945) found that a pH of 5.0 was optimal for the protease activity of both germ and patent flour extracts; and the concentrations of hexylresorcinol and sodium fluoride used effected 60% and 57% inhibitions, respectively, of the protease in the germ extract. A uniform absorption of 62.5% was used on Curves 1, 2, 3, and 4 (Figure 1) and 69% on Curves 5, 6, 7, 8, and 9 to eliminate any possibility of variation due to changing amounts of buffer. The increased absorption is necessary when using the water extract of germ because of the presence of solids in the extract.

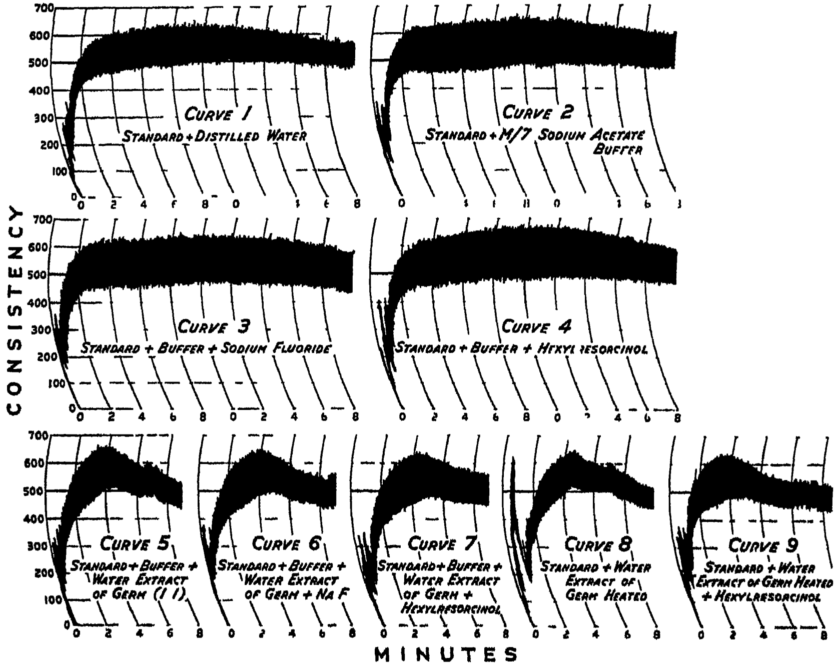


Fig 1 Farinograph curves showing the effect of buffered protease inhibitors with and without germ extract.

Discussion

From Curves 1 and 2 (Figure 1), it is apparent that substitution of water by the particular buffer used, with or without the inhibitors, has little influence on the farinograph curve other than causing a wider amplitude. The water extract of germ had a deleterious effect on the curve. This confirms similar results demonstrated by Sullivan, Howe, and Schmalz (1936, 1937). The addition of the inhibitors to the germ extract did not affect significantly the profile of the farinograph curve. The proponents of the proteolytic theory might claim that the damaging effect of heated germ shown by Sullivan *et al.* (1937) is due to the sulfhydryl content of the extract which could activate the flour protease. However, in Curve 9 it may be seen that, in the presence of hexylresorcinol, the slight improvement produced is about the same as the improvement made by hexylresorcinol on unheated germ extract. This small effect of the hexylresorcinol, therefore, does not seem to be due to its enzyme inhibiting property but rather to some other effect such as that on surface activity.

From the foregoing results it is clear that proteolysis does not seem to explain the effect of the water extract of germ on dough quality. These experiments suggest that the basis of the action of oxidizing and reducing agents must be sought for in some other mechanism.

Summary

The presence of sufficient hexylresorcinol or sodium fluoride to reduce drastically enzyme activity had almost no effect on dough quality as measured by farinograph curves. These findings very strongly indicate that the proteolytic theory of the effect of oxidizing and reducing agents on dough quality is not valid.

Literature Cited

Baker, J. C., Parker, H. K., and Mize, M. D.
1942 The action of an oxidizing agent in bread dough made from patent flours. Cereal Chem. **19**: 334–346.
1943 The pentosans of wheat flour. Cereal Chem. **20**: 267–280.
Balls, A. K., and Hale, W. S.
1936 Further studies on the activity of proteinase in flour. Cereal Chem. **13**: 656–664.
1938 The preparation and properties of wheat proteinase. Cereal Chem. **15**: 622–628.
1940 A sulphur-bearing constituent of the petroleum ether extract of wheat flour. (Preliminary report.) Cereal Chem. **17**: 243–245.
Bungenberg de Jong, H. L.
1938 Proteins in the flour industry. Symposium on proteins. Amsterdam.
Elion, E.
1943 The action of glutathione and wheat germ on dough in relation to proteolytic enzymes in wheat flour. Cereal Chem. **20**: 234–250.
Ford, W. P., and Maiden, A. M.
1938 Effects in dough of glutathione and papain. J. Soc. Chem. Ind. (Transactions) **57**: 278.
Howe, M., and Glick, D.
1945 Activity-pH relationships and inhibition of proteolytic enzyme activity of milled fractions of wheat. Cereal Chem. **22**: 502–513.
Jørgensen, H.
1935 Ein Beitrag zur Beleuchtung der hemmenden Wirkung von Oxydationsmitteln auf proteolytische Enzymtätigkeit: Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. 1. Biochem. Z. **280**: 1–37.
1938 Entwicklung der Theorie der Bromatwirkung, 1935–1938. Muhlenlab. **8**: 201–208.

Olcott, H. S., Sapirstein, L. A., and Blish, M. J.
1943 Stability of wheat gluten dispersions toward reducing agents in the presence and absence of a gluten proteinase. Cereal Chem. 20: 87–97.
Read, J. W., and Haas, L. W.
1939 Studies on the baking quality of flour as affected by certain enzyme actions. VI. Further studies relating to the activation and inhibition of flour proteinase. Cereal Chem. 16: 60–70.
Sandstedt, R. M., and Fortmann, K.
1943 The relation of proteolysis to the characteristics of oxidation and reduction in doughs. Cereal Chem. 20: 517–528.
Sullivan, B., Howe, M., and Schmalz, F. D.
1936 On the presence of glutathione in wheat germ. Cereal Chem. 13: 665–669.
1937 An explanation of the effect of heat treatment on wheat germ. Cereal Chem. 14: 489–495.
——, ——, ——, and Astleford, G. R.
1940 The action of oxidizing and reducing agents on flour. Cereal Chem. 17: 507–528.
Swanson, C. O., and Andrews, A. C.
1945 Effects of papain, yeast water, cysteine, and glutathione on gluten dispersion or on disintegration as indicated by gluten recovery and by mixogram patterns. Cereal Chem. 22: 134–149.
——, and Dines, F. T.
1939 The wheat-meal-time-fermentation test. II. Effect of protease, protease activators, and protease inhibitors. Cereal Chem. 16: 168–177.

ANALYSES OF DOUBLE-CROSS HYBRID CORN VARIETIES PRODUCED ON FARMS

J. J. CURTIS[1] and F. R. EARLE[2]

(Received for publication September 20, 1945)

Corn is the grain crop having the largest planted acreage, the largest bushel production, and the greatest farm value in the United States. Nearly 100 million acres are planted each year, yielding a long-term average of approximately 2½ billion bushels. During the last decade, however, the use of hybrid varieties, with their drought-resistant characteristics and higher-yielding capacities, has raised the average production to about 3 billion bushels. This trend toward the use of hybrids has rapidly and steadily increased until, in 1945, 64% of the total United States acreage and 88% of the Corn Belt acreage was planted with hybrid seed.

Approximately four-fifths of the corn produced in this country is utilized for feed, food, and seed purposes on the farm where produced, while the remainder, or about 500 million bushels, moves into commercial channels. Since the utility of corn either on or off the farm is influenced by its chemical composition, information on the composition is desirable, especially for industrial purposes.

---

[1] Commodity Development Division, Northern Regional Research Laboratory, Peoria, Illinois.
[2] Analytical and Physical Chemical Division, Northern Regional Research Laboratory, Peoria, Illinois.

Essentially all the corn used in industry comes from the Corn Belt, and the general use of hybrid seed in this area focuses attention on the composition of the hybrid varieties now in production. In order to determine the variations which may occur among varieties, samples for analysis were collected from farm fields planted with hybrid corn seed of known variety and source.

Comparatively little information has been published previously concerning the chemical composition of specific corn varieties.

Morrison (1936) reported the average of recent analyses of corn grain and stated that in the development of the present high-yielding varieties of corn there has been an appreciable lowering of the oil content, a slight lowering of the protein, and slight increases in starch and fiber. His data no doubt include both hybrid and open-pollinated varieties.

Ionescu and others (1939) in Roumania found that the starch and oil contents were higher and protein content lower in hybrids than in corresponding pure strains. They found an inverse relationship between protein and starch contents and a direct relationship between the amount of protein and the amount of oil.

Woodworth (1942) reported that selection for high-low chemical composition in a variety of white corn for 41 generations (1896 to 1937) has resulted in low and high strains for oil and protein. The low strains have not been altered much during the last 20 years, but the high strains have shown a consistent increase. During the period from 1934 to 1937, the high protein strain averaged 20.43% protein and the low strain 8.10% protein. During the same period the high oil strain averaged 12.02% oil and the low strain 1.62% oil.

Doty, Bergdoll, and Miles (1943) found that there were no significant differences in the chemical composition of more than 40 commercial hybrids and open-pollinated varieties of dent corn. The composition of yellow varieties tested was practically the same as that of white varieties grown under the same conditions. They determined ash, protein, oil, fiber, and starch.

Materials and Methods

In this paper composition data are presented on 159 samples of hybrid corn of known variety obtained from farm fields in 1941. One hundred and two of these were received at the laboratory as ear corn. For purposes of comparison, similar data also are presented for five hybrid varieties grown at each of three experiment stations.

The collected samples, after drying to a moisture content of about 15% at ordinary room temperature and humidity conditions, were stored at a temperature of 38° to 40°F and a relative humidity of 55 to

60%. Before storage the ear corn samples were shelled and the proportion of cobs to shelled corn determined.

Weight-per-bushel determinations of the shelled corn samples were made following the method prescribed by the Official Grain Standards of the United States. The weight per 1,000 kernels was determined on whole, sound kernels.

All foreign material and damaged kernels were removed from the shelled corn samples before grinding. They were very finely ground in a Bauer mill and analyzed by methods differing from those of the Association of Official Agricultural Chemists only in the following details. Moisture was determined by heating at 130° C for 2 hours, and ash by heating at 550°C for 4 hours. Boric acid was used to receive the ammonia in the Kjeldahl-Gunning-Arnold method for nitrogen. Sugars were run by the Scales' method which has tentative A.O.A.C. approval (A.O.A.C., 1940). Oil was determined by an extraction with petroleum ether in a Butt apparatus (American Oil Chemists' Society method for cottonseed). Starch was determined by the polarimetric method using either the tentative A.O.A.C. method (Lapp, 1944) or

TABLE I

AVERAGE CORNCOB YIELDS OF FARM-GROWN DOUBLE-CROSS HYBRIDS, BY VARIETY AND SOURCE, 1941 CROP

Hybrid variety	Source									Summary		
	Illinois			Iowa			Indiana					
	No. samples	Cob yields per bushel of shelled corn		No. samples	Cob yields per bushel of shelled corn		No. samples	Cob yields per bushel of shelled corn		No. samples	Cob yields per bushel of shelled corn	
		lb	%		lb	%		lb	%		lb	%
U. S. 13	8	10.0	15.1	3	10.3	15.5	6	10.0	15.1	17	10.0	15.2
U. S. 35	5	9.3	14.2	2	10.0	15.2	3	9.7	14.8	10	9.6	14.6
U. S. 44	3	10.1	15.3	5	9.9	15.0	—	—	—	8	10.0	15.1
Ill. 960	2	7.8	12.2	6	9.6	14.6	—	—	—	8	9.1	14.0
Ill. 200	3	10.1	15.3	—	—	—	3	10.9	16.3	6	10.5	15.8
Ill. 21	5	9.3	14.3	—	—	—	—	—	—	5	9.3	14.3
Ill. 784	5	9.4	14.4	—	—	—	—	—	—	5	9.4	14.4
Ill. 877	4	8.7	13.4	—	—	—	—	—	—	4	8.7	13.4
Iowa 13	—	—	—	6	9.3	14.3	—	—	—	6	9.3	14.3
Iowa 939	—	—	—	6	10.6	15.9	—	—	—	6	10.6	15.9
U. S. 63	—	—	—	3	10.1	15.3	—	—	—	3	10.1	15.3
Ind. 608	—	—	—	—	—	—	7	10.2	15.4	7	10.2	15.4
Ind. 610	—	—	—	—	—	—	3	9.7	14.7	3	9.7	14.7
Ind. 613	—	—	—	—	—	—	3	9.3	14.2	3	9.3	14.2
Ind. 844	—	—	—	—	—	—	9	9.6	14.6	9	9.6	14.6
Ind. 416	—	—	—	—	—	—	2	9.3	14.3	2	9.3	14.3
Weighted average	35	9.5	14.4	31	9.9	15.1	36	9.9	15.0	102	9.7	14.8

the Clendenning modification (Earle and Milner, 1944). The specific rotation of starch was considered to be 203°.

While the grain samples from farm fields were not produced under uniform soil and climatic conditions, it is believed that the data presented will give some indication of the composition of the corn produced under ordinary farm conditions. In the case of some of the hybrid varieties it was not practicable to collect a sufficient number of samples for their proper evaluation.

Proportion of Cobs to Shelled Corn

Data on the proportionate yield of cobs to shelled corn for the car corn samples are presented in Table I. These data, obtained by shelling 15-pound samples, show variety averages by states. All ears were well filled and practically free of husks, a condition which seldom occurs commercially. Shelling w[illegible]ished by hand in order to make a perfect separation of grain and cobs. The over-all average shown is 9.7 pounds of cobs per bushel (56 pounds) of shelled corn. The extremes in cob yields of individual samples were 7.8 pounds and 10.9 pounds. The samples from Illinois averaged slightly lower in yield of cobs than those from the other states. Comparison of varieties for all states shows Illinois Hybrid 877 lowest with an average yield of 8.7 pounds and Illinois Hybrid 960, second lowest with an average yield of 9.1 pounds. The varieties with the highest average cob yields were Iowa Hybrid 939 and Illinois Hybrid 200 with yields of 10.6 and 10.5 pounds, respectively.

Chemical Results and Discussion

Analytical data showing variety averages and averages of all shelled corn samples by states are presented in Table II. Results on samples from Ohio and Oklahoma are included in the table, but the number of samples from these states is too small to be representative and they are not considered in the following comparison by states. The Illinois samples averaged lowest in weight per 1,000 kernels and highest in weight per bushel and in oil and sugar contents. The Indiana samples averaged highest in weight per 1,000 kernels and in ash and protein contents, and lowest in starch. The Iowa samples averaged highest in starch content and lowest in ash.

It should be emphasized that some of the differences mentioned in the preceding paragraph and also many of those in the following paragraph are so small that they can be considered only as possible trends. The data presented by Doty *et al.* (1943) indicate that seasonal differences may be even greater than some of the differences mentioned here.

Average analyses of all samples collected for each variety, together with averages of all samples and relative rank of each variety, are presented in Table III. Illinois Hybrid 200 averaged highest in both protein and oil and lowest in starch content. Illinois Hybrid 21 averaged highest in sugar, Illinois Hybrid 960 highest in starch, U. S. Hybrid 44 highest in weight per bushel, and Indiana Hybrid 425 highest in ash content. Iowa Hybrid 13 averaged highest in weight per 1,000 kernels and lowest in protein content. Iowa Hybrid 939 averaged lowest in weight per bushel and in oil content. Illinois Hybrid 877 averaged lowest in weight per 1,000 kernels, Illinois Hybrid 784 lowest in ash content, and U. S. Hybrid 63 lowest in sugar content.

In connection with the data in Table III, it is significant that in every instance the varieties with high protein content had low starch content and vice versa.

The oil content is mainly a varietal characteristic, although some

TABLE II

PHYSICAL AND CHEMICAL COMPOSITION OF FARM-GROWN DOUBLE-CROSS HYBRID CORN VARIETIES, BY VARIETY AND STATE, 1941 CROP

Hybrid variety	No of samples	Air-dry basis		Moisture-free basis				
		Wt. per bushel	Wt. per 1,000 kernels	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
		lb	*g*	%	%	%	%	%
Samples from Illinois:								
U. S. 13	11	60.7	319.3	1.44	10.7	4.7	1.99	69.6
U. S. 35	7	59.3	296.4	1.43	10.7	4.7	1.96	69.6
U. S. 44	4	61.2	308.8	1.43	10.5	4.9	2.17	70.3
U. S. 63	1	59.5	302.0	1.39	10.4	4.7	1.79	70.9
Illinois 21	10	60.1	329.5	1.45	11.4	4.8	2.14	68.9
Illinois 200	8	60.3	308.0	1.46	11.1	5.6	2.14	68.6
Illinois 784	10	60.3	310.6	1.34	10.3	4.9	2.00	69.4
Illinois 877	9	59.9	271.7	1.38	10.6	5.2	1.93	69.7
Illinois 960	5	59.7	298.3	1.43	10.0	4.6	1.93	71.4
Iowa 939	1	57.0	278.4	1.38	9.5	4.0	1.84	71.6
Indiana 416	1	58.3	290.2	1.40	10.2	4.6	1.58	71.9
Indiana 608	1	58.4	304.8	1.48	10.1	4.0	2.03	71.2
Weighted average	68	60.1	305.9	1.42	10.7	4.9	2.02	69.6
Samples from Iowa:								
U. S. 13	3	60.7	305.6	1.29	10.2	4.6	1.85	71.4
U. S. 35	2	59.2	288.6	1.45	9.7	4.4	1.98	71.1
U. S. 44	5	60.5	329.8	1.46	10.5	5.0	2.09	70.4
U. S. 63	5	59.1	281.8	1.36	9.8	4.5	1.87	72.1
Illinois 960	6	60.0	264.7	1.32	10.1	4.6	1.91	71.5
Iowa 13	7	59.5	338.8	1.46	9.8	4.2	2.13	71.8
Iowa 939	8	58.2	343.6	1.36	11.3	3.8	1.94	70.3
Indiana 608	1	58.3	286.5	1.28	10.4	4.0	2.05	71.3
Weighted average	37	59.4	312.1	1.38	10.3	4.4	1.98	71.2

TABLE II—*Continued*

Hybrid variety	No. of samples	Air-dry basis		Moisture-free basis				
		Wt. per bushel	Wt per 1,000 kernels	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
		lb	*g*	%	%	%	%	%
Samples from Indiana:								
U. S. 13	5	59.8	324.8	1.43	10.5	4.6	1.93	70.1
U. S. 35	3	59.7	302.3	1.45	10.9	4.8	2.02	69.4
Illinois 200	3	60.1	334.0	1.49	12.6	5.3	2.06	67.6
Iowa 939	1	58.2	315.0	1.37	11.2	4.0	2.03	70.7
Indiana 416	6	60.9	285.1	1.55	11.3	4.8	1.93	69.2
Indiana 425	1	58.9	323.1	1.59	11.1	4.9	1.91	69.2
Indiana 610	5	58.7	333.0	1.44	11.5	4.8	2.06	69.0
Indiana 613	4	58.6	300.0	1.43	10.8	4.1	1.98	70.6
Indiana 608	9	58.8	304.4	1.44	11.2	4.4	2.00	69.8
Indiana 844	12	59.5	329.8	1.41	11.2	4.6	1.96	69.5
Weighted average	49	59.4	315.2	1.45	11.2	4.6	1.98	69.5
Samples from Ohio:								
Indiana 416	1	58.2	312.5	1.29	10.9	4.8	1.81	69.7
Indiana 425	1	59.5	294.1	1.48	11.1	4.7	1.84	70.1
Indiana 610	1	57.0	313.0	1.44	10.8	4.9	1.93	69.7
Weighted average	3	58.2	306.5	1.37	10.9	4.8	1.86	69.8
Samples from Oklahoma:								
U. S. 35	2	54.4	265.8	1.58	11.3	4.4	1.98	68.9
All samples (159), all varieties, all locations:								
Average		59.6	309.7	1.42	10.8	4.7	1.99	70.0
Maximum		62.5	392.2	1.67	14.1	5.7	2.50	73.4
Minimum		53.1	224.3	1.20	8.3	3.6	1.58	65.8

variation may be attributed to other causes. Only one sample of Illinois Hybrid 200 out of 11 analyzed was below 5% in oil content, while the highest out of 10 samples of Iowa Hybrid 939 had only 4.1% oil. Of the 19 samples of U. S. Hybrid 13 analyzed, 15 were within 0.2% of its average. Illinois Hybrid 200 was outstandingly higher in oil content, and Iowa Hybrid 939 outstandingly lower than the other varieties.

Starch, sugar, ash, and protein may be varietal characteristics, but more data will need to be accumulated over a number of years before definite conclusions can be drawn.

Analytical Data from Hybrid Varieties Produced at Experiment Stations

Samples of five of the double-cross hybrid varieties collected from farm fields also were obtained from the Illinois, Iowa, and Indiana

TABLE III

PHYSICAL AND CHEMICAL COMPOSITION OF FARM-GROWN DOUBLE-CROSS HYBRID CORN VARIETIES, VARIETY AVERAGES FOR ALL LOCATIONS, 1941 CROP

Hybrid variety	No. of samples	Air-dry basis				Moisture-free basis									
		Wt. per bushel		Wt. per 1,000 kernels		Ash		Protein (N × 6.25)		Oil		Sugar		Starch	
		lb	*rank*	*g*	*rank*	%	*rank*	%	*rank*	%	*rank*	%	*rank*	%	*rank*
U. S. 13	19	60.5	2	317.3	7	1.42	11	10.5	12	4.7	9	1.95	11	70.1	7
U. S. 35	14	58.7	13	292.9	13	1.46	4	10.7	10	4.6	10	1.98	8	69.8	9
U. S. 44	9	60.8	1	320.4	6	1.44	7	10.5	12	5.0	3	2.13	2	70.3	6
U. S. 63	6	59.2	11	285.2	15	1.37	14	9.9	16	4.5	13	1.86	17	71.9	2
Illinois 21	10	60.1	6	329.5	5	1.45	6	11.4	2	4.8	5	2.11	1	68.9	16
Illinois 200	11	60.3	3	315.1	8	1.47	3	11.5	1	5.5	1	2.12	1	68.3	17
Illinois 784	10	60.3	3	310.6	9	1.34	17	10.3	14	4.9	4	2.00	7	69.4	11
Illinois 877	9	59.9	7	271.7	17	1.38	13	10.6	11	5.2	2	1.93	13	69.7	10
Illinois 960	11	59.9	7	280.0	16	1.37	14	10.0	15	4.6	10	1.92	11	72.0	1
Iowa 13	7	59.5	9	338.8	1	1.46	4	9.8	17	4.2	15	2.13	2	71.8	3
Iowa 939	10	58.1	17	334.2	2	1.37	14	11.1	5	3.9	17	1.94	12	70.5	5
Indiana 416	8	60.2	5	289.2	14	1.50	2	11.1	5	4.8	5	1.88	15	69.6	12
Indiana 425	2	59.2	11	308.6	10	1.54	1	11.1	5	4.8	5	1.88	15	69.7	10
Indiana 608	11	58.7	13	304.5	11	1.43	9	11.0	8	4.4	14	2.01	6	70.0	8
Indiana 610	6	58.4	16	329.7	4	1.44	7	11.4	2	4.8	5	2.04	5	69.1	15
Indiana 613	4	58.6	15	300.0	12	1.43	9	10.8	9	4.1	16	1.98	8	70.6	4
Indiana 844	12	59.5	9	329.8	3	1.41	12	11.2	4	4.6	10	1.96	10	69.5	13
Weighted average (159)		59.6		309.7		1.42		10.8		4.7		1.99		70.0	

Agricultural Experiment stations. The data for these samples are presented in Table IV, together with data of samples of the same varieties collected from farm fields in the states specified. The experiment station samples, except for Iowa Hybrid 939 grown in Illinois, were consistently lower in ash and protein content, and higher in starch content than were the farm-produced samples, but in other factors there were no consistent differences.

Summary

The ash, protein, oil, sugar, and starch contents were determined for 17 hybrid corn varieties (159 samples) grown in farm fields in 1941, and for five of these varieties grown at the Iowa, Illinois, and Indiana Agricultural Experiment stations.

Variations were found among samples for all chemical constituents for which analyses were made. These differences can be attributed to heredity and/or environment. Oil content was largely a varietal characteristic. In every instance, samples having a high protein content were low in starch content and vice versa. Ash and protein contents were lower and starch higher in experiment station samples than in those grown on farms.

TABLE IV

PHYSICAL AND CHEMICAL COMPOSITION OF DOUBLE-CROSS HYBRID CORN VARIETIES—FARM AND EXPERIMENT STATION SAMPLES, 1941 CROP

Hybrid variety and source	Kind of production	No. samples analyzed	Air-dry basis		Moisture-free basis				
			Wt. per bushel	Wt. per 1,000 kernels	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
			lb	*g*	%	%	%	%	%
U. S. 13									
Illinois	Farm field	11	60.7	319.3	1.44	10.7	4.7	1.99	69.6
Illinois	Experiment station	3	57.5	308.1	1.26	10.6	4.7	2.01	71.6
Iowa	Farm field	3	60.7	305.6	1.29	10.2	4.6	1.85	71.4
Iowa	Experiment station	3	60.6	314.3	1.28	9.5	4.7	2.02	71.7
Indiana	Farm field	5	59.9	320.0	1.45	10.4	4.7	1.93	70.3
Indiana	Experiment station	3	60.5	295.1	1.26	9.4	4.7	2.08	71.8
U. S. 44									
Illinois	Farm field	4	61.2	308.8	1.43	10.5	4.9	2.17	70.3
Illinois	Experiment station	3	60.0	305.7	1.31	9.8	5.2	2.02	72.2
Iowa	Farm field	5	60.5	329.8	1.46	10.5	5.0	2.09	70.4
Iowa	Experiment station	3	60.7	303.7	1.47	10.0	4.9	2.21	74.2
Illinois 784									
Illinois	Farm field	10	60.3	310.6	1.34	10.3	4.9	2.00	69.4
Illinois	Experiment station	3	57.8	306.0	1.29	9.8	5.3	3.19	70.5
Iowa 939									
Illinois	Farm field	1	57.0	278.4	1.38	9.5	4.0	1.84	71.6
Illinois	Experiment station	3	59.0	324.8	1.36	11.2	5.3	2.09	70.6
Iowa	Farm field	8	58.2	343.6	1.36	11.3	3.8	1.94	70.3
Iowa	Experiment station	3	58.7	306.2	1.30	9.8	3.9	1.85	72.3
Indiana	Farm field	1	58.2	315.0	1.37	11.2	4.0	2.03	70.7
Indiana	Experiment station	3	58.6	315.7	1.32	9.6	4.0	2.08	71.6
Indiana 884									
Indiana	Farm field	12	59.5	329.8	1.41	11.2	4.6	1.96	69.5
Indiana	Experiment station	3	58.6	315.7	1.32	9.6	4.0	2.08	71.6

Acknowledgment

The authors wish to acknowledge the assistance of R. M. Glassco, T. A. Scott, R. W. VonKorff, and J. E. Hubbard in the analysis of these samples.

Literature Cited

Association of Official Agricultural Chemists.
, 1940 The official and tentative methods of analysis (5th ed.), p. 499.
Doty, D. M., Bergdoll, Merlin S., and Miles, S. R.
1943 The chemical composition of commercial hybrid and open-pollinated varieties of dent corn and its relation to soil, season, and degree of maturity (a preliminary report). Cereal Chem. 20: 113–120.
Earle, F. R., and Milner, R. T.
1944 Improvements in the determination of starch in corn and wheat. Cereal Chem. 21: 567–575.
Ionescu, Marcea V., Popescu, O., Slusanchi, H., and Goal, L.
1939 Chemical investigation of the Roumanian corn crop of 1938. Anale. Inst. Cercetari Agron. Romaneii II: 266–312 (abstract only available).

Lapp, M.
1944 Changes in official and tentative methods of analysis. J. Assoc. Official Agr. Chem. 27: 87–88.
Morrison, F. B.
1936 Feeds and Feeding (20th ed.). The Morrison Publishing Co., Ithaca, New York.
Woodworth, C. M.
1942 High low chemical strains well established in white corn. Fifty-first Annual Report of Illinois Agricultural Experiment Station for year ending June 30, 1938: 47–48.

LOAF VOLUME POTENTIALITIES, BUFFERING CAPACITY, AND OTHER BAKING PROPERTIES OF SOY FLOUR IN BLENDS WITH SPRING WHEAT FLOUR[1]

KARL F. FINNEY[2]

(Received for publication October 4, 1945)

It has long been recognized that soybean proteins effectively supplement wheat proteins in animal nutrition and greatly increase their nutritive value by the addition of lysine and other amino acids in which wheat proteins are low. Recently much attention has been given to the possibility of improving the nutritive value of bread, macaroni, and other cereal products by supplementing with small amounts of soybean flour. These efforts have met with limited success, the major objections to the use of soy flour in bread being the undesirable bean flavor, the color imparted to the crumb, and the low loaf volume. Considerable work has been done by Beckel and Smith (1944) in overcoming the first two objections.

It seemed probable that loaf volumes might be improved if more was known concerning the baking properties of soybean-wheat flour mixtures. Accordingly, studies with such mixtures or blends have been carried out in the Federal Soft Wheat Laboratory at Wooster, Ohio. This paper describes certain baking properties of these blends and shows how loaf volume and crumb grain are affected when certain alterations in the baking formula and techniques are made on the basis of these properties.

Materials and Methods

Two hexane-extracted soy flours (No. 1 and No. 2) and one extracted with alcohol (A), provided by the Northern Regional Research

---

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Oil and Protein Division, Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture; and the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associate Chemist, Division of Cereal Crops and Diseases, Federal Soft Wheat Laboratory, Wooster, Ohio.

Laboratory, were blended in varying percentages with a commercially milled and bleached hard red spring wheat flour containing 15.0% protein. The blends contained 2, 4, 6, and 8% of soy flour. Each of these and wheat flour alone were baked with 0, 1, 2, 3, and up to 8 mg of potassium bromate per 100 g of flour. Additional ingredients used in all bakes were 6 g sugar, 1½ g salt, 3 g shortening, 2 g yeast, ¼ g malt syrup (120°L), and water as needed. Milk solids were included in the studies with two of the soy flours. An optimum mixing time with the straight-dough procedure and a 3-hour fermentation at 30°C were employed. All punching and panning were performed mechanically. Baking time was 25 minutes at 221°C. Bakings were replicated at least twice. A third replicate was made when loaf volumes differed by more than 25 cc.

Experimental Results

The absorption, mixing time, and the potassium bromate requirement for the wheat flour and the various blends with soy flour are shown in Table I and the loaf volumes, crumb grain scores, and crumb color scores are shown graphically in relation to the potassium bromate requirements in Figures 1 to 3. The bromate requirement given in Table I is the amount of potassium bromate required to produce approximately the maximum loaf volume as shown in Figure 1.

The wheat flour without soy flour and without milk solids produced the largest loaves (Figure 1A) with about 1 mg of potassium bromate. When this flour was blended with various quantities of soy flour, however (Figure 1B to 1M), and baked into bread by the same formula (1 mg of bromate and no milk solids) the loaf volumes were in most instances reduced. For example, 6% and 8% of soy flour No. 1 reduced the loaf volume about 25 cc and 45 cc, respectively, and the same concentrations of alcohol-extracted soy flour (A) depressed loaf volumes 65 cc and 85 cc, respectively. The reductions from the use of soy flour No. 2 were even greater.

The decrease, in general, varied with the amount of soy flour used. With additional increments of potassium bromate, however, the loaf volumes increased to an optimum that was either approximately equal to or considerably greater than that for wheat flour alone, depending on the quantity and kind of soy flour used. Thus, with no milk solids in the formula, the addition of 2% of soy flour No. 1 (Figure 1B) resulted in a loaf volume increase of 70 cc when the quantity of bromate was increased from 1 to 3 mg. Additional amounts of soy flour No. 1 up to the maximum of 8% used in this study also produced loaves larger than those obtained from wheat flour alone, provided additional quantities of potassium bromate were

TABLE I

ABSORPTION, MIXING TIME, AND POTASSIUM BROMATE REQUIREMENT FOR HARD RED SPRING WHEAT FLOUR DOUGHS CONTAINING VARYING AMOUNTS OF SOY FLOURS, WITH AND WITHOUT NONFAT MILK SOLIDS

Kind and percent of soy flour in blends	Baking absorption		Mixing time		Potassium bromate requirement	
	No milk solids	4% nonfat milk solids	No milk solids	4% nonfat milk solids	No milk solids	4% nonfat milk solids
	%	%	*min.*	*min*	*mg*	*mg*
No. 1						
0	68.5	70.5	$3\frac{1}{8}$	$3\frac{1}{8}$	1	1
2	70.5	72.5	$3\frac{1}{8}$	$3\frac{1}{8}$	2	3
4	72.5	74.5	$3\frac{3}{8}$	$3\frac{3}{8}$	3	5
6	74.5	76.5	$3\frac{5}{8}$	$3\frac{5}{8}$	4	5
8	76.5	78.5	$3\frac{7}{8}$	$3\frac{7}{8}$	5	4
A						
2	72.0	74.0	$3\frac{1}{8}$	$3\frac{1}{8}$	1	3
4	75.5	77.5	$3\frac{3}{8}$	$3\frac{3}{8}$	2	3
6	79.0	81.0	$3\frac{5}{8}$	$3\frac{5}{8}$	3	3
8	82.5	84.5	$3\frac{7}{8}$	$3\frac{7}{8}$	3	3
No. 2						
2	71.5	—	$3\frac{1}{8}$	—	2	—
4	74.5	—	$3\frac{1}{4}$	—	2	—
6	77.5	—	$3\frac{3}{8}$	—	3	—
8	80.5	—	$3\frac{1}{2}$	—	4	—

used. The amount of bromate required for approximately optimum loaf volume increased, in general, with the concentration of soy flour. Thus, 5 mg were required for 8% of soy flour No. 1 in contrast to only 1 mg for wheat flour alone. The requirements for the blends of all three soy flours are given in Table I.

Another property of soy flour equally as obvious and striking as "bromate requirement" and "loaf volume imparting potentialities" is the capacity to buffer the oxidative effects of overbromating. All three of these properties are associated with nonfat milk solids, as illustrated by the data in Figure 1A, and are important in test baking and most commercial shop practices. A comparison of these two materials, therefore, is pertinent to a proper evaluation of soy flour. The buffering effect of the latter is well illustrated by the no-milk blend containing 4% of No. 1 soy flour. The maximum loaf volume was secured with 4 mg of potassium bromate (dots, Figure 1C). It will be noted that 5 and 6 mg reduced the loaf volume even less than did an equal excess of potassium bromate when using 4% of nonfat milk solids (circles, Figure 1A).

The same data show that 4% soy flour No. 1 produced greater loaf volume increases than did an equal quantity of nonfat milk solids,

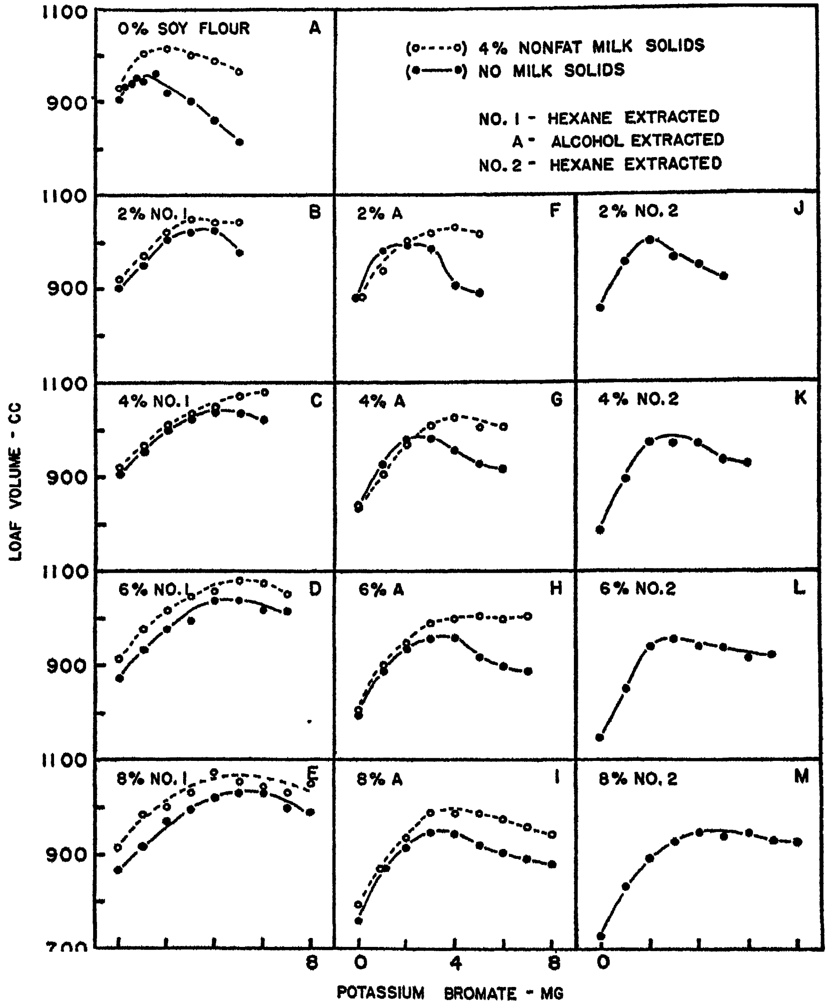


Fig. 1. Loaf volumes for a hard spring wheat flour (15% protein) baked with varying increments of soy flours and potassium bromate, and with and without nonfat milk solids.

provided additional potassium bromate was used. For example, 4% of soybean flour No. 1 increased the loaf volume 80 cc compared to that for the spring wheat flour alone; whereas the same amount of nonfat milk solids (4%) increased loaf volume only about 55 cc, comparisons in both cases being with formulas containing the optimum amounts of potassium bromate. Both the alcohol-extracted and commercial soy flour No. 2 had somewhat the same properties as did soy flour No. 1, but their effects in the blends were less, both in regard to increasing the loaf volume and to buffering effects.

When 4% of nonfat milk solids was used with the soy flour–wheat flour blends (circles, Figure 1B to 1I), further substantial increases in loaf volume resulted in all instances, again provided sufficient potassium bromate was used. This additive behavior of the two materials is also evidenced by the more highly buffered curves, for the most part, and the increased bromate requirements (Table I).

The crumb grain curves (Figure 2) are principally of interest in showing whether the increased loaf volumes of Figure 1 were secured

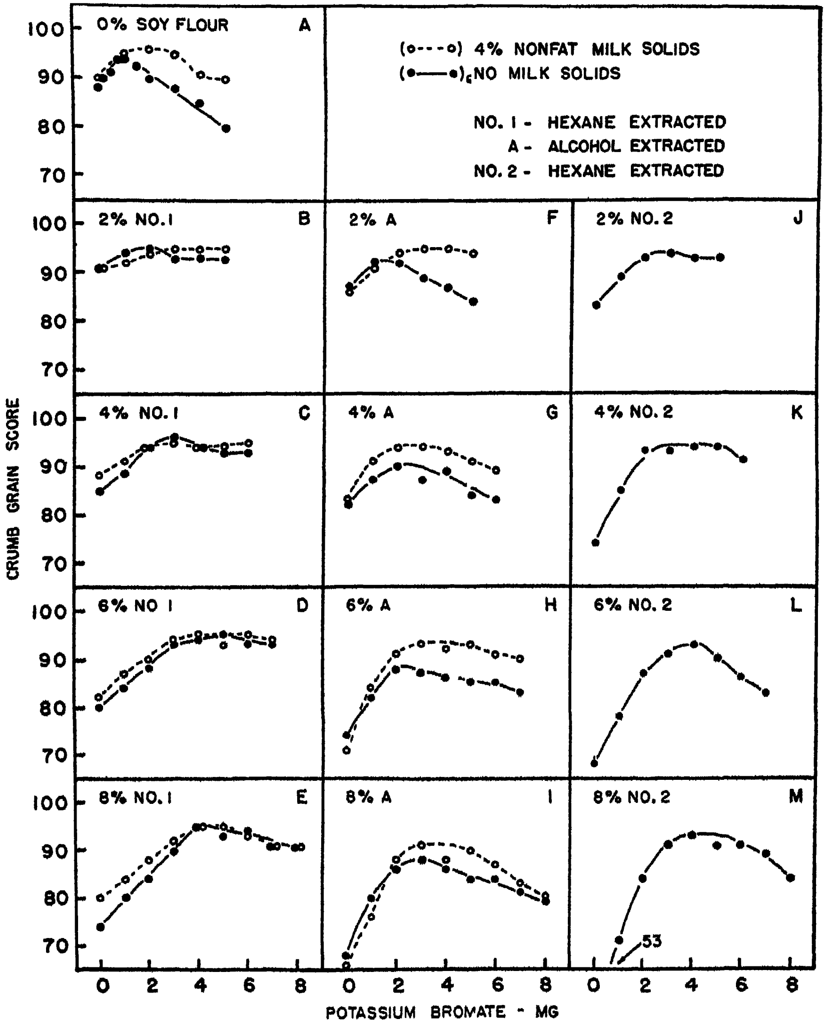


Fig. 2. Crumb grain scores for a hard spring wheat flour (15% protein) baked with varying increments of soy flours and potassium bromate, and with and without nonfat milk solids.

at the expense of the crumb grains of the finished loaf. It will be noted that adding each of the soybean flours decreased the crumb grain scores as compared with the wheat flour alone when only 1 mg of potassium bromate was used, and this depressing effect, also noted for loaf volume, increased with the concentration of soybean flour. These scores, however, gradually improved as the quantity of bromate increased, in the same manner as was noted for loaf volume, so that the loaf volume curves and crumb grain curves roughly parallel each other. Most important, the optimum crumb grains and optimum loaf volumes were produced with the same quantity of bromate in the formula. In addition, the optimum crumb grains for the blends with soy flour No. 1 were fully as good as those obtained with wheat flour alone. In brief, the effects on crumb grain scores are so similar to those on loaf volume that the latter may for all practical purposes be used alone to evaluate these effects. It should be pointed out, nevertheless, that with no more than 4% of the alcohol-extracted soy flour and no nonfat milk solids, most crumb grains are either of questionable quality or are unsatisfactory. With milk solids, however, the crumb grains for all percentages of alcohol-extracted soy flour were considerably improved and satisfactory with the possible exception of the 8% blend. In commercial bread production, therefore, it appears that this alcohol-extracted type of soy flour should be used in conjunction with milk solids.

The crumb color scores corresponding to the optimum loaf volumes and crumb grains for the various blends of soy and wheat flour are shown graphically in Figure 3.

The crumb colors for 2% of soy flour No. 1 without milk solids are detectably whiter than for the wheat flour alone, probably because of the higher volumes which are accompanied by a somewhat finer crumb grain structure. With concentrations greater than 2% the crumb color became progressively more creamy-gray to dull-brownish with increasing concentration of soy flour, each 2% corresponding to an average decrease of 8 points in color score. The effect of the alcohol-extracted flour is about the same and that of the commercial soy flour No. 2 is somewhat greater.

With the scoring used in this study a crumb color value of about 100, which characterizes the control, is considered excellent. It is questionable whether a crumb color of 90 would be noticed by the average consumer of white bread and scores as low as 75 to 80 would go unnoticed by many. Even if observed, many would not object so long as the bread was properly developed and had a suitable texture and crumb grain.

The water absorption and mixing times for the hard red spring

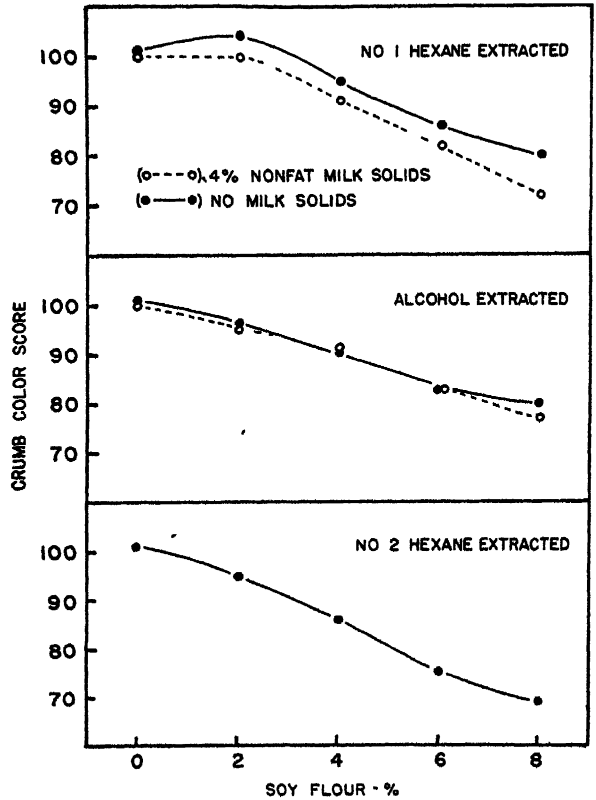


Fig 3. Crumb color scores for a hard spring wheat flour (15% protein) baked with varying increments of soy flours and with and without nonfat milk solids

flour alone and when blended with the various percentages of the different soy flours both with and without milk solids are given in Table I. It was found necessary to add 1.0, 1.75, and 1.5% additional water for each percent of soy flour No. 1, A, and No. 2, respectively.

The first 2% of soy flour added did not alter mixing time appreciably. For each succeeding 2% of soy flour added, an increase of ¼ minute was required except for soy flour No. 2 which required an increase of only ⅛ minute.

General Discussion

The results obtained in this study indicate not only that excellent bread may be made from wheat-soy flour blends including up to 8% of the latter but that loaf volumes and texture-qualities on which consumer preference seems largely to depend may actually be improved. To secure this desirable result, however, it is necessary to use larger quantities of potassium bromate in the baking formula than is customary. In general, the amount of bromate required increases with the percentage of soy flour in the blend. In these studies the bromate requirement for blends including 8% soy flour varied from three to five times that needed for the wheat flour alone.

These results also indicate that soy flour may be used in place of nonfat milk solids and have about the same buffering effect on potassium bromate.

The superior baking properties and potentialities of the hexane-extracted soy flour No. 1 suggest that the solvent and/or extraction technique are of extreme importance in the processing of soybeans into flour for blending with wheat flours, assuming, of course, that the soybeans used in the processing of all three flours were of comparable quality.

The work reported herein should be repeated with other types of flour, particularly hard red winter. If a hard winter flour of comparable protein quality and quantity was used, there seems to be no reason to anticipate results substantially different from those shown with the hard spring wheat flour used in this study, except that the quantity of bromate for optimum results probably would be increased by 1 or 2 mg because of the greater average bromate requirement of the hard winter wheats.

Another potentially important result of this study may be in suggesting a sound procedure for testing the effect of soy flour on bread quality and especially the relative value of different soy flours to be used for this purpose. There is reason to believe that unfavorable results in the past with wheat-soy flour blends may have been due to inadequate baking formulas, especially in regard to the amount of potassium bromate. It seems also that the effect of soy flour on absorption and mixing time must be considered if dependable results are to be secured.

Summary

A commercially milled and bleached hard red spring wheat flour containing 15% protein was baked with various increments up to 8% of each of three different soy flours. Various increments of potassium bromate up to 8 mg per 100 g of flour were used. Absorption and mixing time were varied to suit the needs of each blend. Bakings for two of the soy flour blends were made with and without 4% milk solids.

Excellent bread can be made with blends containing up to 8% soy flour with hard red spring wheat flour, the quality of the bread as measured by loaf volume and crumb grain being even better than for

wheat flour alone provided the quantity of potassium bromate used in the baking formula is increased according to the amount of soy flour used. Absorption and mixing time increased with the amount of soy flour used.

A hexane-extracted flour buffered the effects of excess potassium bromate fully as effectively as nonfat milk solids. The similarity of soy flour to milk solids is of significance from the standpoint of baking technology.

Crumb color of the bread, in general, became progressively more creamy-gray with increasing amounts of soy flour. The color change produced by adding as much as 4% of soy flour probably would not be noticed by most consumers, and would not be objectionable to many others.

Of the three soy flours used in this study a hexane-extracted flour proved the best. In making the comparison, however, the taste and flavor factors were not considered. Soy flours have specific baking properties and potentialities in blends with wheat flour. These potentialities remain hidden, however, unless suitable alterations are made in the baking formula and technique.

Literature Cited

Beckel, A. C, and Smith, A. K.
1944 Alcohol extraction improves soyflour flavor and color. Food Industries 16: 616, 664.

EFFECT OF YEAST, BROMATE, AND FERMENTATION ON BREAD CONTAINING SOY FLOUR [1]

E. G. BAYFIELD and EMERY C. SWANSON

Kansas Agricultural Experiment Station

(Received for publication July 30, 1945)

The proteins of the soybean are high in lysine, and the addition of soy flour to white flour improves the quality of the flour proteins (Carlson *et al.*, 1946; Harris *et al.*, 1944; Jones and Divine, 1944; Volz *et al.*, 1945). But the addition of soy flour to bread without other change in formula or handling is often accompanied by a deterioration in the quality of the bread volume, external appearance, grain, and texture and color of crumb.

The addition of any nonglutinous substance to a dough acts as a diluent of the gluten. This effect, using starch, was studied by Johnson and Bailey (1925). Bailey, Capen, and LeClerc (1935) noted an acceleration of fermentation when soy flour was added to a dough. Bohn and Favor (1945) reported the effects of soy flour on the absorption, mixing tolerance, dough fermentation, gas production, and staling of bread. They found that full-fat soy flour had a water absorption of about 85% and extracted soy flour of 110% when used in doughs. Low-fat soy flour and, to a lesser degree, full-fat soy flour reduced the mixing time, as well as the dough stability upon overmixing. With 3% additions of soy flour, the effect on gas production was negligible. Soy flour acted on a fermenting dough similarly to glutathione. Dough properties were improved by the use of sufficient oxidizing agents, such as bromate and iodate. Soy flour retarded the staling of bread.

---

[1] Contribution No. 120, Department of Milling Industry, Kansas Agricultural Experiment Station.

Some of the factors which may be varied in breadmaking are the fermentation time and the percentage of yeast and potassium bromate. The object of this work was to observe the effects of variations of these factors on the baking properties of wheat- and soy-flour mixtures.

Materials and Methods

Full-fat and low-fat (both extracted and expeller) soy flours were obtained from the five largest producers. The analyses of these were very uniform in each class.

These samples were given preliminary baking tests by the standard A.A.C.C. procedure. It was found that full-fat and extracted soy flours had very similar baking characteristics. Expeller soy flours showed inferior baking characteristics. Composition of the representative low-fat (extracted) soy flour selected for study was: moisture, 7.0%; ether extract, 1.0%; protein (N × 6.25), 51.0%; ash, 5.5%.

The wheat flour was a standard patent Kansas flour of 0.48% ash and 13.0% protein from the 1943 crop. All doughs were made up using 6% sugar, 1.5% salt, and 0.25% 120°L malt. The absorption was that found to be optimum by the mixograph. Immediately after mixing, 170 g of dough was scaled. Punching and molding were performed by machine to obtain uniformity. Low form pans were used. The volume and weight of the loaves were taken immediately after removal from the oven. Texture, grain, and crumb color were scored the following day.

Potassium bromate was varied in amounts of 0, 1, 3, 5, 7, 9, and 11 mg per 100 g flour and 1%, 2.5%, and 4.0% yeast were used. The percentages of soy flour were 0, 1.5, 3.0, 4.5, 6.0, and 7.5. The fermentation times (to molding) were 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hours.

Three variations of yeast, six of soy flour, six of bromate, and seven of fermentation make a total of 756 possible variations. Since the work was performed in duplicate, a total of 1,512 loaves was produced.

Straight doughs were used in this work. While it is realized that most commercial bread is made by the sponge method, it is fairly generally agreed that indications given by the straight dough method on the effects of variations in bromate, fermentation time, etc., apply to sponge doughs, when properly interpreted (Shellenberger and Ziemke, 1939).

To determine the effect of dilution of the gluten by soy flour on bread volume, the standard A.A.C.C. method was used. In control loaves 3, 6, and 9% of starch were used and compared to the same quantities of low-fat (extracted) and full-fat soy flours.

The bread was scored for the customary factors. The volume of loaves was reported in cubic centimeters. Crumb texture was scored on the basis of silkiness and compressibility. A standard (optimum) texture was scored 95. Textures under 80 were poor, being harsh and open, with round, thick cell walls. While the word "open" usually refers to grain rather than texture, all texture scores under 80 indicated a poor grain. Grain was scored in connection with texture. A standard grain was scored 2.00, a close grain 3.00, and an open grain 1.00. Grain scores were not too significant, but are shown in Table IV, which gives averages of all the data obtained. Fermentation and baking loss were obtained by weighing 170 g of dough out of the mixer, and weighing the bread as it came from the oven.

Discussion of Results

Figure 1 shows the effect of gluten dilution on volume. Soy flour reduces the volume of bread more than can be accounted for by

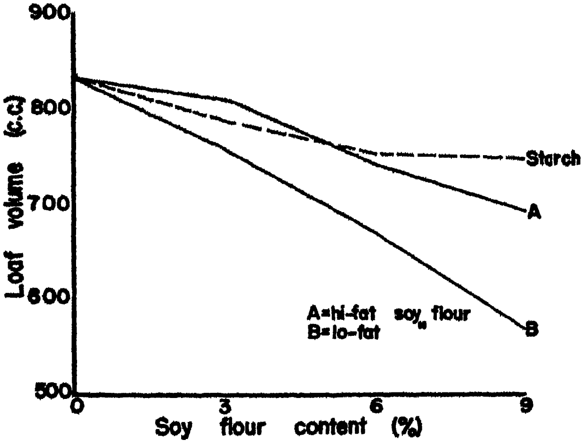


Fig. 1. Effect of soy flour on loaf volume.

straight dilution. This would indicate that soy flours contain some factor which has a deteriorating effect on bread volume.

Figure 2 shows visually the effect of some of the variables studied on loaves containing 9.0% soy flour. This is a higher level than was used in this study, and higher than would be used in commercial white bread. Even with this high percentage of soy flour, bread made under optimum conditions closely resembles the check (nonsoy) loaves.

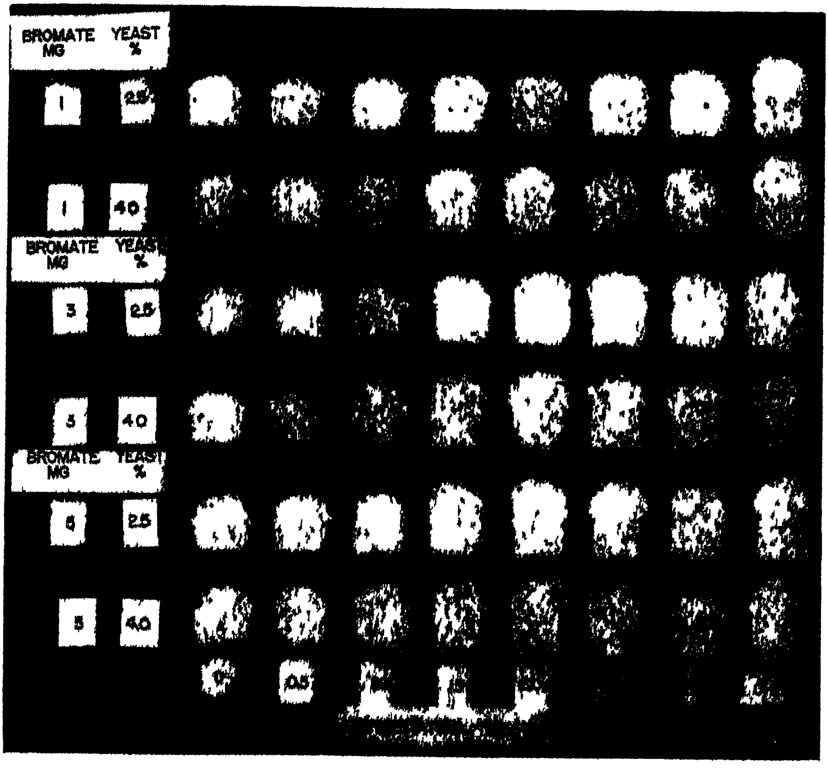


Fig. 2. Internal characteristics of typical loaves from formula procedure study—9.0% soy flour used in all but the check loaves.

It would be too cumbersome to present the data on all the loaves produced. For brevity in Tables I, II, and III, the extremes of bromate used have been omitted, along with the data for some of the intermediate fermentation times and soy flour percentages.

The data in these tables cover the range of the optimum conditions for soy flour within the limits of practical, commercial formulas.

Effect of Soy Flour on Loaf Volume. For optimum volume, bread containing 1.5% of soy flour requires about 2 mg more bromate per

TABLE I

EFFECT OF VARIATIONS OF SOY FLOUR, BROMATE, AND FERMENTATION WITH 1% YEAST ON BAKING PROPERTIES

Fermentation time	Baking property	No soy flour			3% soy flour			6% soy flour		
		Mg bromate			Mg bromate			Mg bromate		
		3	5	7	3	5	7	3	5	7
1 hr.	Volume cc	555	492	535	455	472	512	428	455	465
	Texture	50.0	48.5	68.5	54.0	61.0	50.0	45.0	46.5	40.0
	Ferm. and bake loss %	10.9	10.7	12.2	11.4	10.7	11.1	10.7	10.6	10.7
2 hr.	Volume cc	572	598	620	532	565	592	492	570	522
	Texture	82.5	76.5	75.5	82.0	78.5	81.5	67.5	73.0	49.0
	Ferm. and bake loss %	12.2	12.8	12.1	12.8	11.8	12.7	10.9	11.9	11.7
3 hr.	Volume cc	666	693	665	648	640	660	602	613	580
	Texture	86.0	87.0	72.5	85.0	79.0	70.0	81.5	78.5	60.0
	Ferm. and bake loss %	13.6	13.9	14.2	13.6	13.2	12.2	12.9	12.3	13.7

TABLE II

EFFECT OF VARIATIONS OF SOY FLOUR, BROMATE, AND FERMENTATION WITH 2.5% YEAST ON BAKING PROPERTIES

Fermentation time	Baking property	No soy flour			3% soy flour			6% soy flour		
		Mg bromate			Mg bromate			Mg bromate		
		3	5	7	3	5	7	3	5	7
1 hr.	Volume cc	670	780	725	595	648	665	565	612	640
	Texture	89.0	91.5	85.5	59.5	77.0	90.5	55.0	71.0	78.0
	Ferm. and bake loss %	13.4	15.9	14.4	12.8	13.1	14.5	12.2	12.6	12.1
2 hr.	Volume cc	842	860	820	748	800	778	692	790	732
	Texture	87.0	90.0	89.0	72.5	92.0	80.0	81.5	88.0	88.0
	Ferm. and bake loss %	15.7	15.4	16.0	15.4	16.1	15.5	14.5	15.4	14.2
3 hr.	Volume cc	745	736	722	772	730	710	738	738	705
	Texture	81.0	84.0	82.5	78.0	85.0	71.5	85.5	86.5	82.0
	Ferm. and bake loss %	15.9	15.9	15.5	16.5	15.8	15.4	15.1	16.0	15.7

100 g flour than nonsoy bread. Higher quantities of soy flour require additional increments of bromate. Loaf volume is also increased with shorter fermentation times, if yeast and bromate are increased to the optimum. With optimum yeast, bromate, and fermentation time,

TABLE III

EFFECT OF VARIATIONS OF SOY FLOUR, BROMATE, AND FERMENTATION WITH 4% YEAST ON BAKING PROPERTIES

Fermentation time	Baking property	No soy flour			3% soy flour			6% soy flour		
		Mg bromate			Mg bromate			Mg bromate		
		3	5	7	3	5	7	3	5	7
1 hr.	Volume cc	822	892	892	748	815	892	638	830	825
	Texture	94.5	91.0	68.5	82.0	94.0	97.0	78.5	87.5	92.0
	Ferm. and bake loss %	16.2	16.8	17.9	14.3	15.7	16.7	13.5	15.4	16.2
2 hr.	Volume cc	840	912	822	872	845	842	845	822	838
	Texture	94.0	89.5	92.5	86.5	95.0	93.5	89.5	81.0	88.5
	Ferm. and bake loss %	17.2	17.2	16.9	16.2	16.7	16.3	16.6	16.4	16.9
3 hr.	Volume cc	765	708	665	778	725	742	782	752	675
	Texture	74.5	66.5	87.0	85.5	84.0	89.0	90.0	77.5	86.0
	Ferm. and bake loss %	19.0	16.3	16.4	17.0	16.8	17.1	16.3	17.4	15.6

bread containing up to 6% of soy flour was approximately equal in volume to the control (nonsoy) bread.

Effect of Soy Flour on Crumb Texture. For optimum texture, extra bromate and shorter fermentation are required where soy flour is used. As is usually the case, the conditions which produce optimum loaf volume are similar to those which give the best texture.

Effect of Soy Flour on Fermentation and Baking Loss. There was a significant lowering of fermentation and baking loss in doughs containing soy flour. This is best shown in Table IV, where the mean values of all of the data are given.

The data show that by relatively minor modifications of fermentation time and bromate level, bread containing 3% of soy flour can be made which compares closely to nonsoy bread. With greater changes in fermentation and bromate, bread of good volume, grain, and texture can be made with 6% added soy flour.

Color of Crumb was not scored individually in these studies because it was early found that the following conditions persisted throughout the whole of the work:

With 3% soy flour and optimum conditions the color of crumb was practically as good as the control. With 6%, the color of crumb was darker, although under optimum conditions even 6% soy flour makes a loaf of bread which would probably be considered quite satisfactory for white bread by the consuming public.

Statistical Studies

The mean values for the effects of the various factors are shown in Table IV. These figures were obtained by averaging all of the results for each factor.

TABLE IV

MEAN VALUES OF MAIN EFFECTS

(All doughs scaled 170 grams immediately after mixing)

Main effect	Loaf volume	Texture score	Grain score	Loaf weight	Fermentation and baking loss
	cc			g	%
Yeast (%)					
1.0	503	65	1.84	150.4	11.5
2.5	659	71	2.00	146.8	13.7
4.0	726	74	1.89	145.1	14.7
Soy flour (%)					
.0	659	73	2.00	146.8	13.7
1.5	652	72	1.99	147.2	13.4
3.0	643	71	2.05	147.3	13.4
4.5	626	70	2.02	147.5	13.3
6.0	610	68	2.04	147.8	13.1
7.5	587	66	1.95	148.1	12.9
Bromate (mg)					
1	586	69	2.00	148.7	12.5
3	623	70	2.13	147.5	13.3
5	645	72	2.02	147.2	13.4
7	643	71	1.80	147.2	13.4
9	640	70	1.66	147.4	13.3
11	640	70	1.61	147.4	13.3
Fermentation time (hr.)					
.0	440	60	1.93	151.2	11.1
0.5	544	65	2.00	149.3	12.2
1.0	639	70	2.08	147.9	13.0
1.5	702	72	2.04	146.7	13.7
2.0	707	75	2.04	146.2	14.0
2.5	694	72	2.01	145.5	14.4
3.0	680	72	2.00	144.9	14.8

Figure 3 shows graphically the effect of the various interactions of soy flour, bromate, fermentation time, and yeast on volume and texture.

Table V shows the results of variance analysis for loaf volume data, and the covariance analyses for texture and loss during fermentation and baking. It was found that all of the factors studied had a significant effect on the loaf volume. All of the first order interactions were significant with respect to volume. The data also indicated a significant interrelationship for all four of the factors studied in regard to volume.

After the influence of loaf volume on texture was removed by covariance analysis it was found that none of the factors studied in-

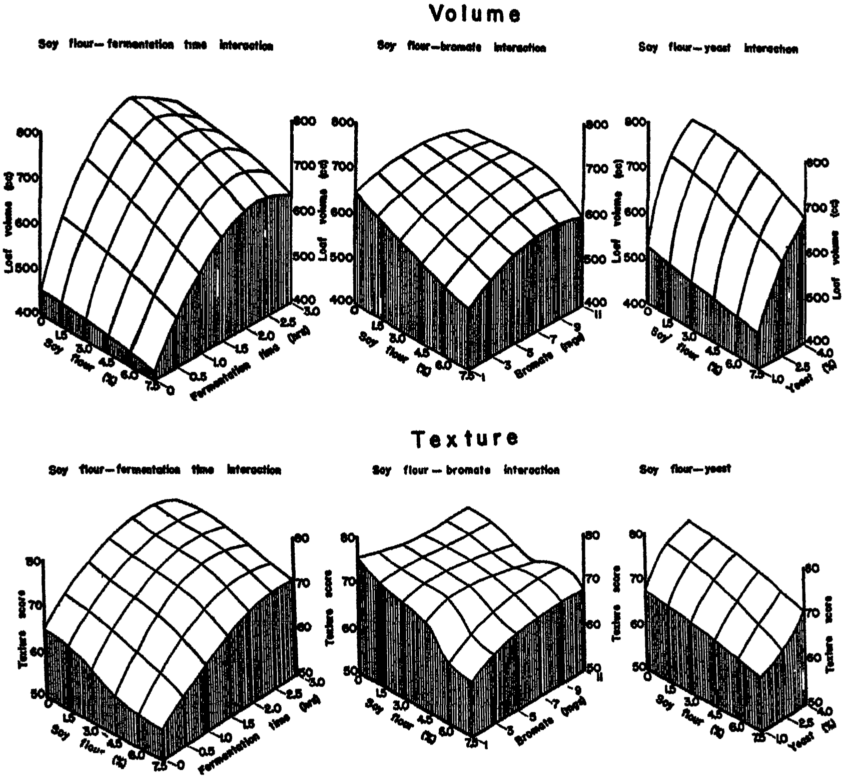


Fig. 3. Volume and texture interactions of formula procedure study.

dependently influenced the texture. However, the first order interactions indicated a significant interrelationship with respect to their influence on texture.

After the influence of loaf volume on the fermentation and baking losses was removed by covariance analysis it was found that percentage of soy flour and fermentation time were of significant influence. The first order interactions were all significant, with the exception of the soy flour-fermentation time interaction.

Summary

With 3% to 6% added soy flour in straight doughs, it was necessary to increase potassium bromate and decrease fermentation in order to produce bread of optimum volume, grain, and texture. For optimum results with 1.5% soy flour 2 mg extra bromate was approximately the best amount. Larger percentages of soy flour required increasing amounts of bromate. Even with as much as 6% soy flour, proper balancing of the factors of yeast, bromate, and fermentation produced

TABLE V

VARIANCE ANALYSES OF THE DATA FOR VOLUME, TEXTURE, AND FERMENTATION AND BAKING LOSS

Source of variation	Volume (variance)		Texture (covariance)		Fermentation and baking loss (covariance)	
	d.f.	Mean square	d.f.	Mean square	d.f.	Mean square
Yeast	2	6,560,632**	2	17	2	145.0
Bromate	5	130,092**	5	260	5	7.8
Soy flour	5	191,861**	5	56	5	20.6*
Fermentation time	6	2,211,680**	6	563	6	187.0*
Interactions						
Yeast × bromate	10	9,095**	10	573**	10	5.0**
Yeast × soy flour	10	11,485**	10	313**	10	5.7**
Yeast × Ferm. time	12	161,399**	12	650**	12	64.2**
Bromate × soy flour	25	6,907**	25	210**	25	5.4**
Bromate × Ferm. time	30	25,224**	30	243**	30	3.9**
Soy flour × Ferm. time	30	8,550**	30	119*	30	1.7
Higher interactions	620	2,763**	1,375	80	620	2.4
Duplicate error	756	710			755	2.1

* Significant at 5% point.
** Significant at 1% point.

bread equal to the control (nonsoy flour) bread except for crumb color. Relatively small changes in these factors were required to produce standard white bread when 3% soy flour was used.

Soy flour reduced the fermentation and baking loss, in proportion to the amount added.

Acknowledgment

Acknowledgment is made to the Soy Food Research Council of the Soy Flour Association, Chicago, for its cooperation and financial assistance in carrying out this project. The assistance of Ralph M. Bohn and Larry Trempel in the preparation of this article is gratefully acknowledged.

Literature Cited

Bailey, L. H., Capen, R. G., and LeClerc, J. A.
1935 The composition and characteristics of soybeans, soybean flour, and soybean bread. Cereal Chem. 12: 441–472.
Bohn, R. T., and Favor, H. H.
1945 Functional properties of soya flour as a bread ingredient. Cereal Chem. 22: 296–311.
Carlson, S. C., Hafner, F. H., and Hayward, J. W.
1946 Effect of soy flour and milk solids in white bread on the nutritive value of its protein. Cereal Chem. (in press).
Harris, R. S., Clark, M., and Lockhart, E. E.
1944 Nutritional value of bread containing soya flour and milk solids. Arch. Biochem. 4: 243–247.
Johnson, A. H., and Bailey, C. H.
1925 Gluten of flour and gas retention of wheat flour doughs. Cereal Chem. 2: 95–106.

Jones, D. B., and Divine, J. P.
1944 The protein nutritional value of soybean, peanut, and cottonseed flours and their value as supplements to wheat flour. J. Nutrition 28: 41–49.
Shellenberger, J. A., and Ziemke, W. H.
1939 A critical study of a "pup" sponge baking method. Cereal Chem. 16: 32–35.
Volz, F. E., Forbes, R. M., Nelson, W. L., and Loosli, J. K.
1945 The effect of soy flour on the nutritive value of the protein of white bread. J. Nutrition 29: 269–275.

BOOK REVIEWS

Methods of Analysis of the American Society of Brewing Chemists. Fourth Revised Edition. Prepared by Technical Committee and Editorial Board, Louis Ehrenfeld, Editor, 1944. Copies can be obtained from Mrs. Hilda Holmes, Executive Secretary, Route No. 4, Sawyer, Door County, Wis., at the following prices for non-members: Methods $3.50; Extract Tables $2.50; Beer and Wort Tables $2.50.

The present edition in a paper-covered, loose leaf binding represents an extensive expansion and revision of the previous edition. In addition to revised and enlarged sections on Malt, Cereal Adjuncts, Brewing Sugars and Syrups, and Beer, new sections are included on Hops, Wort, Yeast, and Spent Grains.

In the first section on malt, the major changes are an alternative method for bushel weight, employing a 250 ml graduated cylinder and a special funnel, and the ferricyanide modification for the determination of diastatic power. Methods for moisture, extract, and color in caramel malt and for moisture and color in black malt are included for the first time.

The section on brewing sugars and syrups includes for the first time methods for the determination of hydrogen ion concentration (pH) and total reducing sugars. A rapid fermentation method (4–5 hours) is given for fermentable extracts. The Fehling's solution procedure only is given for the determination of diastatic power of malt syrups. The ferricyanide modification should be as suitable for this material as for malt if a blank determination is carried out on each sample and the necessary corrections made in the calculation.

To the section on beer has been added methods for the determination of dextrins and calibration of hydrometers. These additions complete a very comprehensive collection of methods for the analysis of beer.

The new section on hops includes methods for sampling, physical examination, moisture, and resins. Ten methods which are in common use for the analysis of wort are combined in a new section. A method for total solids in liquid and pressed yeast is given in the section on yeast. To complete the book a section on spent grains gives procedures for moisture, available extract, and soluble extract and one is referred to the A.O.A.C. for feed analysis methods.

The third edition of the tables for Extract Determination in Malt and Cereals represents an enlargement of the previous edition to include higher specific gravities and higher moisture contents. These changes make the tables more suitable for determination of yield of extract in cereals.

The tables relating to wort, beer, and brewing sugars and syrups are a corrected reprint of the earlier edition except for Table 4, "Reducing Sugar Values by the Munson and Walker Method," which has been greatly expanded. This booklet contains tables for Extract in Wort and Beer, Alcohol, Correction Factors for Original Extract, Reducing Substances (Munson-Walker), Reducing Substances (Lane-Eynon), and Baume Degrees as related to extract content.

The book of methods and accompanying tables is a very complete and useful publication especially for brewery, brewing, and malting laboratories.

ALLAN D. DICKSON
University of Wisconsin
Madison, Wisconsin

Physical Methods of Organic Chemistry. I. Edited by Arnold Weissberger, Eastman Kodak Company. Interscience Publishers, Inc., New York, N. Y. 1945. 736 pp., 15 × 23 cm. Price $8.50.

The purpose of this book, as stated in the preface, is to provide an adequate description of important physical methods that are of proven usefulness to chemists. The object of the editor and the authors, who are experts in the method each describes, is to present the techniques in sufficient detail together with enough of the theoretical background so that those who are interested in their use may be saved the tedious and oftentimes difficult job of assembling the "know how" from periodicals and specialized books.

The methods discussed in this volume, 16 in number, together with the authors and the number of pages devoted to each are as follows: I. Determination of Melting and Freezing Points—E. L. Skau and H. Wakeham (46 pp.). II. Determination of Boiling and Condensation Temperatures—W. Swietoslawski (22 pp.). III. Determination of Density—N. Bauer (38 pp.). IV. Determination of Solubility—R. D. Vold and M. J. Vold (28 pp.). V. Determination of Viscosity—H. Mark (14 pp.). VI. Determination of Surface and Interfacial Tension—W. D. Harkins (53 pp.). Including a discussion of the Parachor—G. W. Thomson (9 pp.). VII. Determination of Properties of Monolayers and Duplex Films—W. D. Harkins (42 pp.). VIII. Determination of Osmotic Pressure—R. H. Wagner (24 pp.). IX. Determination of Diffusivity—A. L. Geddes (34 pp.). X. Calorimetry—J. M. Sturtevant (124 pp.). XI. Microscopy—E. E. Jelley (96 pp.). XII. Determination of Crystal Form—M. A. Peacock (30 pp.). XIII. Crystallochemical Analysis—J. D. H. Donnay (24 pp.). XIV. X-Ray Diffraction—I. Fankuchen (36 pp.). XV. Electron Diffraction—L. A. Brockway (32 pp.). XVI. Refractometry—N. Bauer and K. Fajans (84 pp.).

Of particular advantage to the uninitiated is the aim of some of the authors to evaluate critically the various modifications of the method under discussion, pointing out the specific advantages or shortcomings of each modification and the conditions under which each may be expected to yield the most dependable results. Proper interpretation of results is stressed and illustrated by examples in many cases. Apparatus to be employed is described by diagrams and detailed discussion. Numerous literature citations appear along with the text and references of a general character are included at the end of each chapter.

The usefulness of this volume is by no means restricted to organic chemists or even to chemists actively engaged in research. Advanced students will find the critical descriptions of practical methods helpful to their understanding of the theoretical aspects involved.

The editor and publishers have done a commendable job. There are few errors.

D. R. Briggs
Division of Agricultural Biochemistry
University of Minnesota
St. Paul 8, Minnesota

CEREAL CHEMISTRY

VOL. XXIII MARCH, 1946 No. 2

A MACHINE FOR MEASURING THE EXTENSIBILITY AND RESISTANCE TO EXTENSION OF GLUTEN[1]

K. HLYNKA and J. ANSEL ANDERSON

Grain Research Laboratory, Board of Grain Commissioners for Canada,
Winnipeg, Manitoba

(Received for publication September 20, 1945)

The physical properties of gluten are important in breadmaking and have been widely studied, but there is as yet no entirely satisfactory and generally accepted method of measuring them. It is true that any experienced cereal chemist can make an excellent estimate of the quality of the gluten washed from a dough by manipulating it in his hands; the difficulties are that the judgment is subjective and depends on the experience of the chemist, and that the resulting opinion cannot be readily recorded or transmitted to another person. There have been a number of attempts to overcome these difficulties by devising machines for measuring certain properties of gluten. These machines and related ones for measuring the physical properties of dough have been reviewed by Bailey (1940), and two other machines have since been reported.

Kress (1924) described one of the earliest machines for testing gluten and James and Huber (1927) used it for measuring the properties of glutens washed from different samples and subjected to various treatments. Mohs, Schmidt, and Frank (1939) reported a technique for use with the Brabender glutograph, which draws a "glutogram" representing extension of the gluten and the force required to extend it until it breaks. A device for measuring the flow and spring of crude gluten was described by Baker, Parker, and Mize (1942); and Baker, Mize, and Parker (1943) presented data obtained with a machine which measured the force necessary to penetrate a gluten ball held in a special holder.

The apparatus described in this paper measures the extensibility of crude gluten and its resistance to extension. It has been called a

---

[1] Published as paper No. 80 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, and as paper No. 243 of the Associate Committee on Grain Research (Canada).

"Stretchometer." The design presents nothing fundamentally new since it is similar in many respects to the Brabender glutograph and to some other apparatus. However, for various reasons it seemed advisable to design and build a new machine rather than make a close copy of an old one. This paper reports on the development of a standard technique for use with the Stretchometer, on the application of the machine to studies of gluten quality, and on the limitations encountered in these studies.

Apparatus and Methods

The Stretchometer. A photograph of the Stretchometer is shown in Figure 1 It consists of a spring balance (covered by a face-plate *A*)

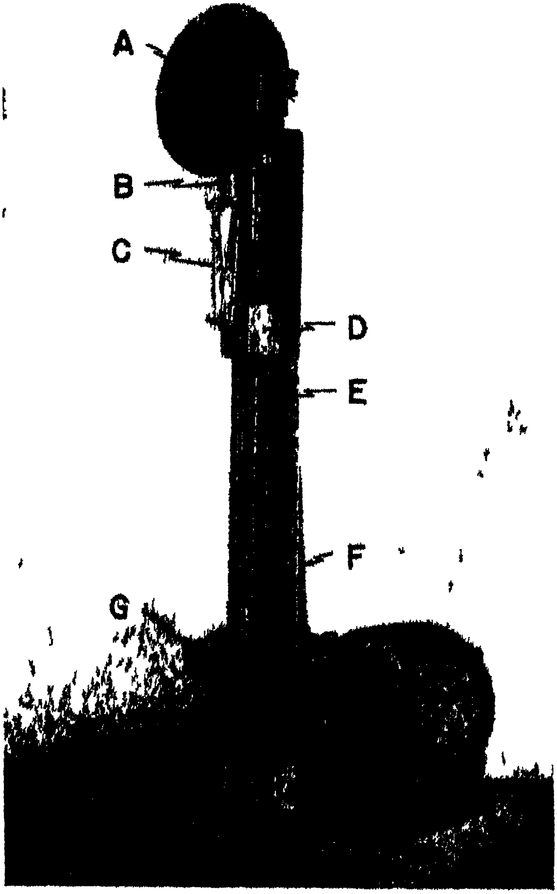


Fig. 1. Gluten Stretchometer

supported at the top of a vertical frame *F* on which a block *D* is moved by the screw *E*, which is driven by a motor operating through the gear box *G*.

A ring of gluten is hung over an inverted Tee *B* suspended from the balance, and a removable pin is passed through the two loops of gluten *C*, and inserted into the block *D*. When the motor is started, the four strands of gluten are stretched simultaneously. The distance that the gluten stretches before breaking is noted from the position of the pointer on the vertical scale on the right, and the resistance to extension is measured by the balance. The vertical scale is graduated from 0 to 40 cm, and the balance from 0 to 150 g.

A drawing of the balance assembly at the top of the Stretchometer is shown in Figure 2. The balance is made from a clock spring, with its inner end fastened to a spindle mounted on ball bearings, and the outer end attached to the frame. The glass Tee, on which the gluten is hung, is attached to a thin metal band wound around a one-half inch boss on the spindle. An aluminum disc attached to the front end of the spindle has a scale calibrated in grams, and a pawl engaging with notches on the edge of the disc holds it in the maximum position after the weight is removed when the gluten breaks. Readings are made through a window in the face plate.

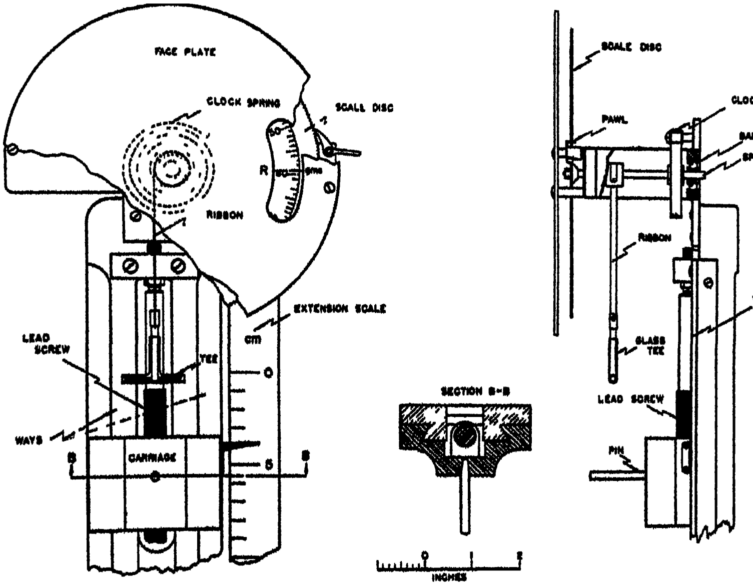


Fig. 2. Partial detail of Stretchometer assembly.

The main stand was cast in bronze from a pattern made in the laboratory, and the ways and other parts were subsequently machined. The base of the stand was designed to fit the top of the inverted gear box *G*, salvaged from an old Hobart dough mixer, which serves as a reducing gear. The operating screw *E* is set into the vertical shaft in the gear box and is held at the top by a pivot thrust bearing. It has 20 threads to the inch and is so made that the block carriage *D* can run off the thread at the top to set itself automatically at zero, and, as a safety device, can also run off at the bottom. The machine is driven by a reversible motor so that the block can be returned to the starting position after each test. Motor and V-belt drive were so selected that the block moves at 40 cm per minute when the gear box lever is in the "low" position.

Fig. 3. Equipment for preparing rings of gluten

Other Equipment. For preparing the rings of gluten, pairs of iron plates for freezing gluten discs, and a cutter for cutting the rings were made. The plate assemblies, both open and closed, are shown on the left in Figure 3, and the cutter is shown at the right. The plates are 4 × 5½ inches and are made from 3/16-inch iron. Spacing studs on the lower plate, against which the top plate comes to rest, are so adjusted that a gluten disc with a uniform thickness of 3 mm is produced. The cutter is made from an old embossing stamp and cuts out the rings in one operation. The ring has external and internal diameter of 50 and 35 mm and weighs about 3 g.

Preparation of Sample. The technique finally adopted for use with the Stretchometer is briefly described in this subsection, and the experiments dealing with the effects of varying the conditions for different steps of the technique are described later. For duplicate tests, a dough of baking absorption is prepared by mixing 75 g of flour with water for one minute in the Hobart-Swanson mixer. In experiments made so far, the gluten has been washed out by hand under a small stream of tap water maintained at 25°C. The gluten is divided into two equal portions, which are rounded into balls. Each ball is then placed between a pair of cellulose acetate discs, 0.01 inch thick, and squeezed into a disc by clamping between the plates described above. The gluten is then frozen by placing the plate assembly in a chamber at −20°C for 45 minutes. This is done to facilitate the preparation of the test rings. To accelerate freezing, the plate assemblies are kept in the refrigerator when not in use and are thus precooled.

When the frozen sample is removed from the plates, the cellulose acetate discs peel off readily and the gluten remains frozen long enough for the ring to be cut from it. Before stretching, the rings are conditioned for 30 minutes in water in a small jar placed in a constant temperature water bath at 25°C. Fifty milliliters of water at 30°C are put into the jar with the frozen gluten and the conditioning temperature of 25°C is thus reached almost immediately.

Stretching. The method of making the stretching test will be obvious from the foregoing descriptions of the Stretchometer. Extensibility is measured in centimeters by means of the longitudinal scale, and resistance to extension is measured in grams by the balance.

Other Methods. Fisher and Halton (1936) described two moisture methods for gluten; one involved boiling the gluten before drying in an air oven, and the second made use of specific gravity. Another method has been devised in this laboratory. The surface moisture of the sample tested on the Stretchometer is removed by manipulating it in the hands, and the sample is placed in a 25-ml crucible, covered with a watch glass, and weighed. Enough 95% ethyl alcohol (4 ml) is added to cover the sample and after a few minutes the uncovered crucible is put in an air oven at 130°C. The alcohol causes the gluten to puff up during drying as it does in the vacuum oven, and complete drying is obtained in 3 hours. This method gives the same result and replicability as the vacuum oven method.

The dried samples can be readily crushed into fine powder with a pestle while in the crucible. After redrying the crushed samples for 30 minutes, aliquots of 0.2 g are weighed for Kjeldahl determinations.

Standardization of the Test Procedure

In developing a procedure for use with a new machine, such as the Stretchometer, a number of arbitrary decisions must first be made on the conditions under which each step of the procedure shall be carried out. A large amount of investigation of the effects of varying the conditions and on the precision with which the conditions should be controlled can then be undertaken; but if this is too extensive, the more important task of applying the test is delayed. A compromise must be effected, and in the present instance this involved making limited studies of the conditions for several of the major steps of the procedure. The results are described in the following subsections.

Mixing of Dough. Information was sought on the possible effect on Stretchometer results of the amount of work done on the gluten during mixing. Accordingly, doughs from three different flours were prepared by mixing for 1 and 3 minutes in a Hobart-Swanson mixer, and glutens were washed out and tested. Mean values for eight replications are as follows:

Flour	Gluten properties							
	Moisture content		Protein content		Resistance		Extensibility	
	1 min	3 min	1 min	3 min	1 min	3 min	1 min	3 min
	%	%	%	%	g	g	*cm*	*cm*
1	66.3	67.9	80.4	81.0	67	70	26	26
2	65.8	66.9	81.0	81.4	93	93	24	24
3	65.4	67.0	81.4	80.5	98	96	22	24

The only constant difference between glutens from doughs mixed 1 and 3 minutes is that the latter have higher moisture contents, that is, they showed greater absorptions. But this difference did not affect either extensibility or resistance. Glutens of similar mean protein content were readily obtained irrespective of whether the doughs were mixed for 1 or 3 minutes, and even the small differences in protein content that exist are reflected by parallel differences in the resistance values.

As a smooth dough can be obtained by mixing for 1 minute, this time was selected as standard. Longer mixing, particularly with weaker flours, makes the dough more difficult to handle.

Amount of Washing. A number of studies were made of the effects on gluten of the amount of washing and manipulation it receives while it is being recovered from the dough. The results of studies made with two different flours are shown in Figure 4. Sample 1 was an experimentally milled flour of 12.6% protein, while sample 2 was a commercially milled flour of a similar protein content of 12.7%. Replicate doughs from each flour were washed by one operator for different lengths of time in random order. Washing time was varied between 6 and 32 minutes.

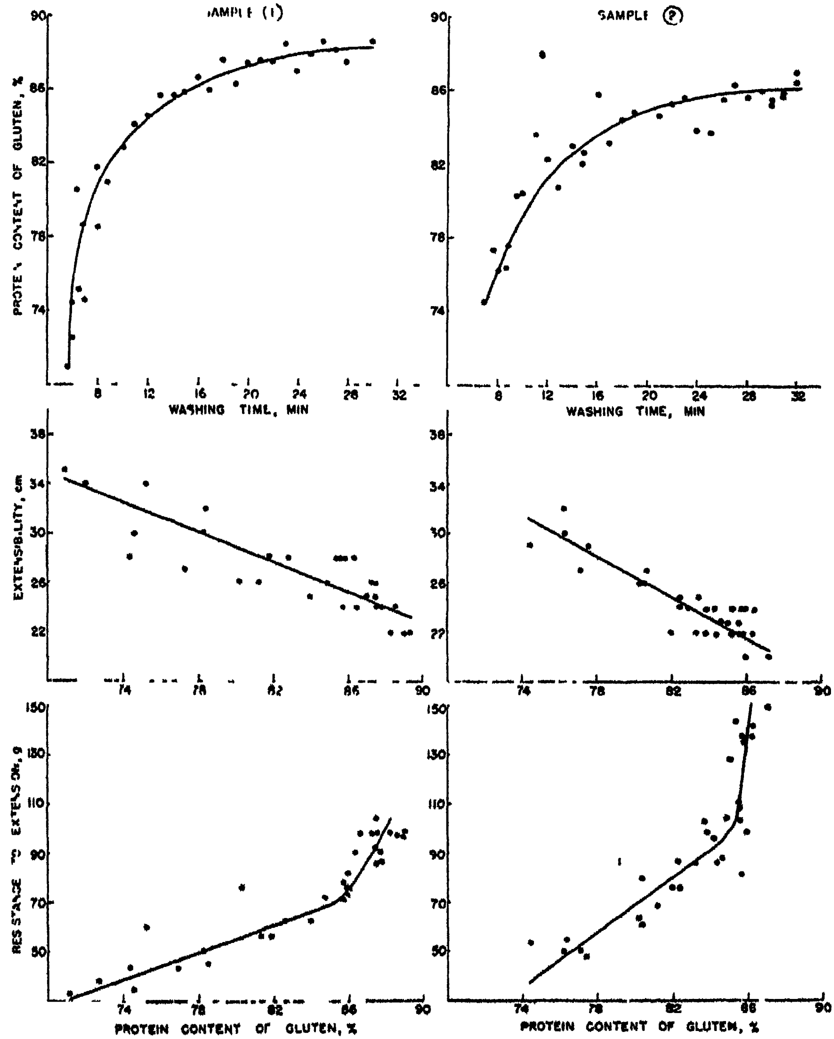


Fig. 4. Relations illustrating effects of washing glutens for different lengths of time.

Relations between washing time and the protein content of the glutens are shown in the top pair of curves. The curves have the expected shape; the protein content of the crude gluten increased rapidly during the early stages of washing and less rapidly as washing progressed. Sample 1 apparently yielded its gluten more readily; it attained a higher final protein content and approached this level more rapidly. The curves also show that Sample 2 was more difficult to handle uniformly during washing.

It seemed best to accept the protein content of the gluten as a criterion of the amount of washing, and the middle pair of curves therefore show extensibility plotted against protein content rather than against washing time. The relation between the amount of washing and extensibility appears to be linear, and differences of about 10 cm in the extensibility of the gluten from a given sample of flour can be obtained by wide variations in the amount of washing. Deviations of the points from the regression lines are almost wholly accounted for by the experimental error, which suggests necessity for replication.

The bottom pair of curves in Figure 4 deal with resistance to extension, and here again the deviations from the curve are accounted for mainly by the experimental error of the Stretchometer measurement. During the first part of the washing period there is a linear relation between protein content and resistance, but towards the end, at about 85% protein, the curve rises sharply so that resistance increases while the protein content of the gluten changes little. For that reason further experiments described in this paper deal with glutens washed to protein contents within the range in which relations are linear.

Freezing. Rapid freezing seemed desirable, and as the lowest temperature that could be conveniently maintained in the freezing cabinet was −20°C, this temperature was adopted for all tests. Mean values for quadruplicate discs frozen for 20, 40, and 60 minutes are given below:

Time *min*	Extensibility *cm*	Resistance *g*
20	25.0	73
40	29.5	78
60	30.0	75

At 20 minutes the gluten did not appear to be fully frozen. Differences between the results for 40 and 60 minutes are within the experimental error and a time of 45 minutes was chosen mainly to suit the testing schedule.

Conditioning Temperature. The influence of the temperature at which the gluten rings are conditioned after freezing was studied by testing duplicate rings from five glutens conditioned for 30 minutes at each of three temperatures of 20°, 25°, and 30°C.

Temperature °C	Extensibility cm	Resistance g	Moisture in gluten %
20	25.1	100	65.8
25	25.4	81	65.8
30	26.1	77	66.0

The mean results show that increasing the temperature increased the extensibility slightly and reduced the resistance to extension. A temperature of 25°C was chosen as standard, and it was obvious that temperature would have to be closely controlled by means of a water bath and thermostat.

Conditioning Time. Duplicate tests were made on five glutens conditioned at 25°C for 20, 40, and 60 minutes.

Time min	Extensibility cm	Resistance g	Moisture in gluten %
20	25.8	89	65.9
40	27.4	87	66.1
60	25.0	80	66.3

Erratic results were obtained for extensibility, but increasing conditioning time reduced the resistance to extension and increased the moisture content of the gluten. These effects are not large and minor errors in timing would not be serious. A conditioning time of 30 minutes was selected as standard.

Uniformity of Sample Rings. The suitability of the cutting technique and the degree of uniformity required in the sample rings was investigated by exaggerating the differences. Rings of different weight were obtained by cutting them from glutens frozen in different thicknesses. Data for the means of triplicate tests are given below.

Weight of ring g	Extensibility cm	Resistance g
2.87	29	63
2.94	30	68
3.04	30	68
3.20	29	73

Extensibility is little affected by increasing the weight of the ring, but resistance increases appreciably. In later experiments, however, it was shown that the standard error for the weight of a single ring is only about ±0.03 g, which is not large enough to have a serious effect on resistance. It appeared that gluten rings weighing about 3 g would be suitable, and space adjustments between plates were thus left at 3 mm.

Replicability of Tests. Statistics on the precision of the Stretchometer and auxiliary tests were obtained from three major experiments. Standard errors of single determinations, calculated from differences between tests made on duplicate rings, are given in Table I.

TABLE I

PRECISION OF STRETCHOMETER AND AUXILIARY TESTS

Property of gluten	Experiment 1 (22 pairs)	Experiment 2 (48 pairs)	Experiment 3 (96 pairs)
Standard errors of single determinations			
Weight of sample ring, g	0.02	0.04	0.03
Moisture content of gluten, %	0.18	0.19	0.18
Protein content of gluten, %	0.35	0.39	0.38
Extensibility, cm	1.4	2.0	2.2
Resistance, g	4.7	8.0	5.0
Coefficients of variability			
Extensibility, %	5.1	8.2	6.9
Resistance, %	7.7	9.3	8.2

Standard errors for weight of rings, moisture, and protein are less than 1%, and are very satisfactory. On the other hand, standard errors for the Stretchometer values are somewhat higher. Differences in the magnitude of the errors of extensibility and resistance measurements for the three experiments are not fully explained by differences in the mean values. This is demonstrated by the coefficients of variability given in the last two lines of the table, which are not much more uniform than the corresponding standard errors.

The failure to obtain better replicability for the Stretchometer measurements is undoubtedly due to the difficulty of preparing duplicate gluten rings that will invariably stretch and break in the same manner. If quadruplicate tests are made, the standard error is reduced by half (i.e., divided by $\sqrt{4}$), and with such replication differences of about 4% in extensibility and resistance can be demonstrated. In practice it has been found that the precision obtained with duplicate tests is adequate for most investigations, because the principal experimental error is in preparing the gluten rather than in testing it.

Application of the Stretchometer to Studies of Gluten Quality

The Stretchometer has been used in studies of differences in gluten quality among a series of samples of Thatcher wheat and among a second series consisting of composite samples of six different varieties. The results throw some light on the inter- and intravarietal relations between Stretchometer measurements and other quality data, and also serve to emphasize some of the difficulties that must be overcome before the Stretchometer can be used as a routine rather than as a research tool.

Thatcher Series. Eight samples of flour, milled from Thatcher wheat, and ranging in protein content from 10.5 to 16.5%, were obtained. For each sample of flour, a series of 12 glutens of increasing protein content were prepared by different amounts of washing. The amount of washing required was judged by weighing the gluten mass from time to time until a predetermined weight was obtained. Extensibility, resistance to extension, moisture content, and protein content were determined for each gluten.

The detailed data for the Stretchometer measurements are shown in Figure 5, in which both extensibility and resistance are plotted against the protein content of the glutens. There are scatter diagrams for each of the eight samples, and one graph in which all the regression lines have been assembled. The scales on the left deal with resistance, as do the black dots and the lower regression lines. The scales on the right, the open circles, and the upper lines deal with extensibility.

The pattern of the black dots in each diagram shows that there is a close relationship between resistance and the extent to which the gluten is washed, as indicated by the protein content of the gluten (cf. Figure 4). The correlation coefficients lie between .82 and .96, with .71 required for a 1% level of significance. The regression coefficients differ considerably. Values for samples 1, 2, and 3 are about 3.9 g for each per cent of protein, whereas those for samples 5, 6, 7, and 8 are about 2.2 g, and the regression coefficient for sample 4 is much lower, 1.6 g. An analysis of residual variance showed that the differences between the regression coefficients were highly significant (found, $F = 4.7$; required for 1% point, $F = 2.9$). The investigation thus shows that the resistance values for glutens from different samples of the same variety do not change by equal amounts when washing produces equal changes in the protein contents of the glutens.

The analysis of residual variance also showed highly significant differences between centroids ($F = 9.4$), that is, between the mean values for resistance. The lower right-hand diagram in Figure 5 certainly shows that samples 1 and 2 have higher resistances than all other samples with the possible exception of sample 4. Indeed, the only sample whose position with respect to the others cannot be determined with certainty is sample 3; but even its resistance is clearly lower than those of samples 1 and 2 and greater than those of samples 6, 7, and 8. On the whole, therefore, the results are quite clear-cut.

The data on extensibility cannot be interpreted as readily as those on resistance. Most of the correlation coefficients for extensibility and protein content were not significant owing to the small change in extensibility for each per cent protein and the somewhat large error in the determination of extensibility. There is considerable crossing among the regression lines, and the analysis of residual variance did not show significant differences between the slopes. Accordingly, it cannot be assumed that the extensibilities of glutens from different samples change at different rates during washing. On the other hand,

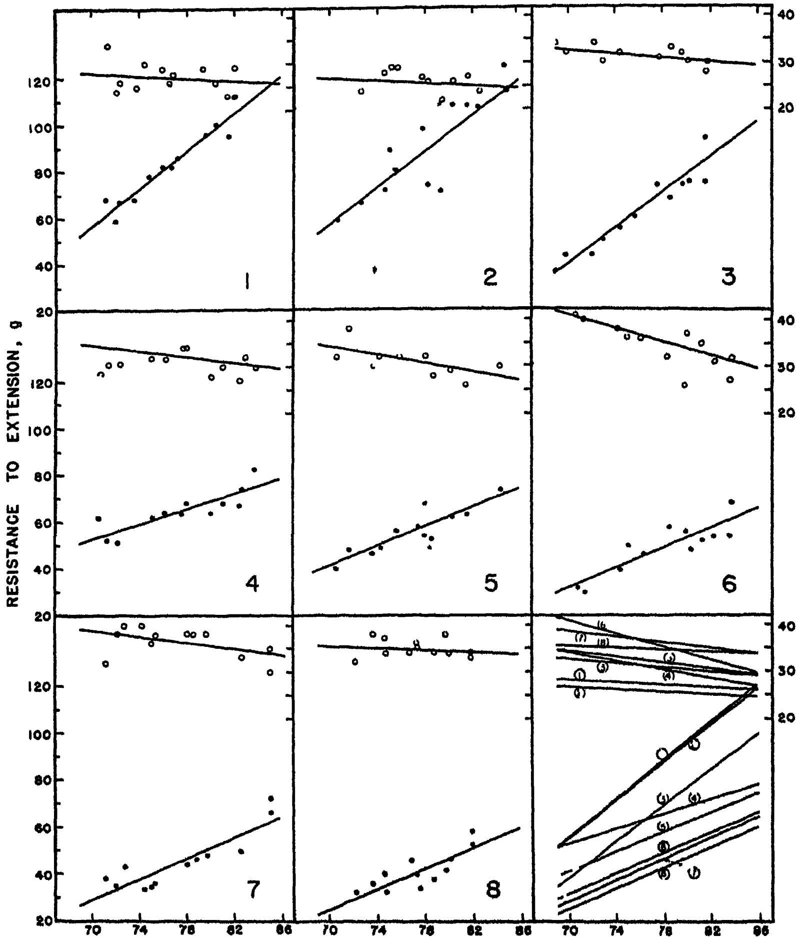


Fig. 5. Relations between protein content of gluten and resistance to extension, and between protein content and extensibility

significant differences between centroids, or mean values, were established ($F = 26.9$). Reference to the upper group of lines in the assembly diagram in Figure 5 will show that there is little doubt that samples 6, 7, and 8 have higher values than samples 3, 4, and 5, and that the latter have higher values than samples 1 and 2. But within each of these groups the samples cannot be differentiated.

In order to examine possible relations between Stretchometer and other values, data on protein content, absorption, and loaf volume by the malt-phosphate-bromate procedure (Geddes, Aitken, and Fisher,

1940) were obtained for the original flours, and Stretchometer values were calculated to a common protein basis. The mean protein content for all glutens from all flours was 77%; and with the aid of appropriate regression coefficients Stretchometer values for each flour sample were calculated for this protein level. These values are reported in Table II together with gluten moistures, mean protein content of the gluten, and baking data.

TABLE II

Stretchometer and Other Data for Thatcher Series

Sample no.	Gluten			Flour		
	Resistance	Extensibility	Moisture	Absorption	Protein	Loaf volume
	g	cm	g	%	%	cc
1	85	28	65.6	60.3	11.8	733
2	84	26	64.9	60.2	13.5	757
3	65	32	65.1	65.6	10.5	630
4	64	32	66.3	62.7	14.6	795
5	56	31	66.4	62.3	16.5	843
6	47	34	67.3	63.7	12.7	796
7	44	36	67.2	63.5	15.1	842
8	40	35	67.7	63.7	16.0	1027

The first two columns of data show that within these samples of one variety there is an inverse relation between resistance and extensibility ($r = -.95$; 1% point is .83). There is also evidence of an inverse relation between resistance and the moisture content of the glutens ($r = -.90$), and, of course, of a direct relation between the latter and extensibility ($r = +.86$). Neither resistance nor extensibility is significantly related to either the protein content or the loaf volume of the flour; and the anomalous absorption for sample 3 interferes with the possible relation between the moisture content of the gluten and the absorption of the flour. Within this series of Thatcher flours, loaf volume appears to depend mainly on the protein content of the flour ($r = .83$). The interpretation of differences between samples as shown by Stretchometer values in Table II is the same as that discussed in dealing with Figure 5.

Intervarietal Series. Stretchometer tests were also made on composite samples of Thatcher, Marquis, and four new varieties (C.T. 141, 149, 508, and 807), representing equal parts of grain from a series of replicated plots grown at six stations in western Canada in 1944. As these samples had been studied as part of another investigation, data were available on baking qualities and on the dough properties measured by the farinograph, extensograph, alveograph, and mixograph. In the present study, the gluten from each sample was tested at nine levels of protein content obtained by the method described in the preceding section Regression lines for extensibility and resistance, each plotted against protein content, are shown in Figure 6 The correlation coefficients for extensibility and protein were not significant, and an analysis of residual variance did not show significant differences either between regressions or centroids. Thus the study failed to demonstrate differences among extensibilities of the glutens from the six varieties.

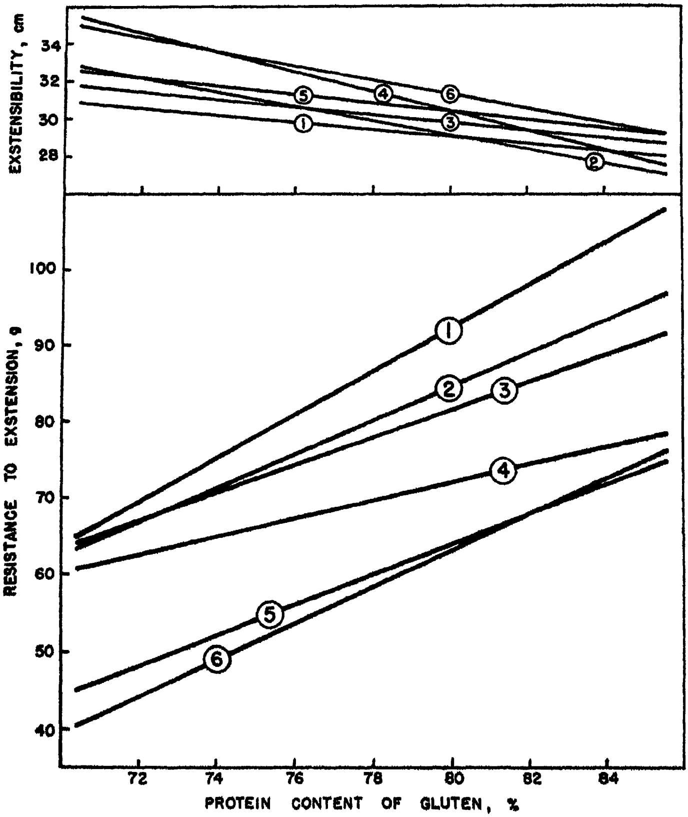


Fig 6 Lines for six varieties showing regression of stretchometer values on protein content

Correlation coefficients for resistance and protein content of the gluten were .70, .75, .87, .80, .92, and .87, with .67 and .80 required for 5 and 1% levels of significance; and the regression coefficients were: 2.86, 2.21, 1.77, 1.10, 1.88, and 2.38. Statistical analysis showed no significant differences between regressions but a highly significant

TABLE III

STRETCHOMETER AND BAKING DATA FOR VARIETAL SERIES

Variety	Gluten		Flour	
	Resistance	Extensibility	Flour protein	Loaf volume
	g	cm	%	cc
1. C. T. 508	88	27	14.2	805
2. Thatcher	82	28	13.6	800
3. C. T. 141	79	28	12.5	735
4. Marquis	70	29	13.0	770
5. C. T. 149	61	29	13.3	780
6. C. T. 807	60	30	13.4	760

difference between centroids ($F = 22.9$; required for 1% level, 3.5). This result would be expected from consideration of Figure 6.

The mean values for the Stretchometer readings were calculated to a common basis of 78.6% protein, which was the general mean for all glutens in this study. The adjusted means, together with data for the protein contents of the original flours and for loaf volume, are given in Table III. In view of the inverse correlation between resistance and extensibility that occurred in the Thatcher series, the corresponding relation shown in Table III is interesting, although no great importance can be attached to it because of the lack of significant differences between extensibility values. There is little evidence of any relation between Stretchometer values and protein content of the flour or loaf volume. In this series again, loaf volume appears to depend mainly on the quantity of protein in the flour.

Figure 7 shows the curves obtained with various machines used for measuring the physical properties of doughs. Normal and high-speed farinograms are shown in the first column, and these are followed by mixograms, extensograms, and alveograms. This figure was prepared for a report which was completed before the Stretchometer study was undertaken. It is therefore of particular interest that the varieties are arranged in exactly the same order as that of Table III, namely, in decreasing order of Stretchometer resistance. Figure 7 shows that this is also the order in which both the farinogram and the mixogram place the varieties with respect to strength. It thus appears that the mixing properties of the doughs are related to the mean resistance values of the glutens.

Resistance to extension is measured for the doughs by the height of the extensogram, while the initial stiffness of the doughs is measured by the height of the alveogram. It might thus be expected that these measurements would be related, at least in part, to the Stretch-

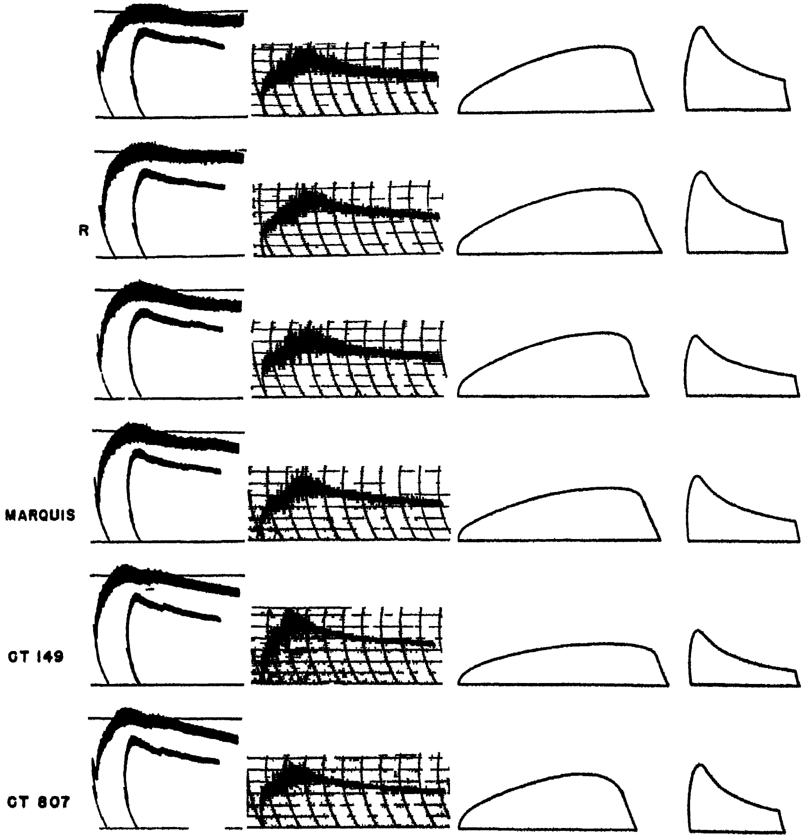


Fig 7 Normal and high speed farinograms mixograms extensograms and alveograms for each of six varieties

ometer resistance Such a relation appears to exist for the first five varieties, but is upset by the values for the sixth Since the Stretchometer failed to differentiate between the extensibilities of the varieties it is hardly surprising that no relation is apparent between the Stretchometer value and extensibility as shown by the lengths of the extensogram or alveogram.

General Discussion

The design of the Stretchometer appears to be very satisfactory and no trouble has been experienced in operating it. However, tests made with glutens reconstituted from dried material, many of which have much greater resistance to extension than freshly prepared gluten, indicate the advisability of providing a balance of larger capacity if a second model were built, though this difficulty can be overcome with the present model by hanging the gluten ring over the Tee in such a way that two rather than four strands of gluten are stretched. Work with rings of dough also suggests that it might be advisable to provide a longer shaft and scale to handle samples which have extensibilities beyond the capacity of the present machine.

When the Stretchometer was built, consideration was given to the advisability of incorporating a recording mechanism so that a curve representing the relation between resistance and stretching time could be drawn. This was not done because it seems to us that the use of a recording mechanism tempts the investigator to undertake complex studies of curve shape, area under the curve, and other characteristics, which are likely to prove fruitless until the significance of the simpler and more readily interpreted measurements of extensibility and resistance to extension is understood.

The technique developed in preparing gluten rings of standard dimensions is simple and reproducible within the limits of sampling error. As freezing can be done on the coils of an ordinary refrigerator, no special equipment is required other than the freezing plates and the ring cutter, and these can be made quite cheaply. The comparable technique used by Mohs, Schmidt, and Frank (1939) with the glutograph involves pretreatment ("homogenization") of the gluten ring which is formed under 25 kg of pressure, in a press designed for the purpose, and held for 30 minutes at 40°C. Before stretching, the ring is cooled for 20 minutes at 18°C. James and Huber (1927) were apparently able to press a satisfactory disc in the Kress (1924) machine after pretreating the gluten by allowing it to stand in water for 10 to 12 hours.

The effect of freezing on gluten properties cannot be readily determined because of the difficulty of preparing rings from unfrozen gluten similar to those made from the frozen gluten. It seems dangerous to assume that this effect will be negligible, although it is probable that it will not be greater than the effects of heating or of allowing the gluten to stand in water for a long period. Nevertheless, the freezing technique must be classed as an arbitrary one adopted to facilitate the test.

The paramount difficulty of comparing the qualities of the gluten in different samples of flour lies in isolating the glutens. It would be ideal if the gluten could be readily obtained in unchanged and "pure" condition, that is, with maximum protein content. But the protein determination takes too long, and there are no other satisfactory criteria for determining when the gluten has been obtained in sufficiently pure form or whether its properties have been changed during extraction. It may well be impossible to obtain pure and unchanged gluten.

The curves in Figure 4, dealing with the effect of continued washing, illustrate the difficulties. Both extensibility and resistance change as the gluten becomes successively purer, that is, as its protein content increases. But the curve for resistance also suggests, by the change in slope towards the end of the washing period, that a stage is reached at about 85% protein when further washing removes little additional nonprotein matter, but continues to increase resistance. And it seems reasonable to suppose that this further change in resistance is caused by the work done during the manipulation of the gluten mass. If this is true, then the question arises as to how much of the increase in resistance (or decrease in extensibility) during the earlier stages of washing results from purification of the gluten and how much results merely from the work done on it.

It might be reasonable to test all glutens at equal but not necessarily maximum protein contents. But here again there are difficulties. Glutens from different flours cannot be brought to the same protein content by washing for equal times since the starch is not released with uniform ease. Accordingly, washing by machine as practiced by Fisher and Halton (1936) would be no great help. Moreover, because different glutens hold different amounts of water, equal final protein contents cannot be obtained by washing to calculated final wet weights. This difficulty could be overcome by pilot tests, though these would be cumbersome. But if all glutens were washed to the same protein content by varying the amount of washing, the work done on the glutens would not be equal and each would probably experience a different amount of change in its physical properties.

These points, which do not appear to have been brought to light in earlier studies, were demonstrated in the present investigation by making comparisons at several different protein contents obtained by washing for different lengths of time. While this technique is by no means ideal, and would hardly prove suitable for a routine test, it does serve to replicate the experiment, to permit comparisons by calculation at a standard final protein content, and to provide additional information on the changes in gluten properties that take place during washing.

Glutens appear to respond differentially to continued washing, and a knowledge of the rate of change of physical properties as washing progresses may be of considerable value. If these changes are brought about partly by the work done on the gluten, they may well be similar to changes that take place in the gluten when a dough is mixed and subjected to other mechanical action during the remainder of the baking procedure. Whether relations of this sort can be demonstrated remains to be seen. In the meantime it seems clear that the differential response of glutens to washing is caused by differences in their physical properties, and that the elaboration of a suitable technique for comparing glutens is no easy task. However, the difficulties created by the differential effects of washing can easily be overemphasized. Changes in the rank order of samples when the testing procedure is varied are a matter of universal experience. It is well known, for instance, that the placing of a series of flours is frequently changed when the baking formula is changed. But if two samples of flour differ widely in baking quality, then this difference will be apparent by all baking methods. Accordingly, a simplified and standardized method for isolating and testing glutens may well prove useful. It should serve to differentiate glutens that differ appreciably in quality, though it may not serve to separate glutens of somewhat similar quality.

The results of the present study seem sufficiently encouraging to warrant further investigation. Standardization of the washing method, by making this more mechanical, is obviously required, and some improvement in the reproducibility of the Stretchometer measurements is desirable. If these can be achieved it should be possible to make considerable progress in studying gluten quality. Whether progress can be made in relating the physical properties of glutens to the baking qualities of flour remains to be seen. One thing seems certain, however; simple relations between the various properties are hardly to be expected, and the need for large scale studies of sufficient size to permit the use of correlation and partial correlation techniques can therefore be anticipated.

Summary

Technique and equipment for measuring the extensibility and resistance to extension of gluten are described. A ring of gluten (internal and external diameter, 35 and 50 mm; weight, about 3 g) is stamped from a pressed disc, 3 mm thick, made manageable by freezing. After thawing in water at 25°C for 30 minutes, the ring is folded to form four strands, suspended from a balance, and stretched downwards by a pin moving at 40 cm per minute. Extensibility of the gluten is measured in centimeters by the distance it stretches before breaking, and resistance is recorded in grams by the balance. The machine is called a Stretchometer. Data showing the effects of changing the conditions for various steps of the testing procedure are reported.

When gluten is washed from a dough its resistance increases and its extensibility decreases with the increasing protein content of the gluten brought about by continued washing. The relation between

resistance and protein content is linear up to about 85% protein where additional washing continues to increase the resistance but removes little more nonprotein matter. The change in gluten properties during washing may result not only by purification but also from the work done on the glutens. The change in extensibility is not as marked as that in resistance, and the relation between extensibility and final protein content appears to be linear throughout.

Glutens were prepared from eight samples of Thatcher wheat flour ranging in protein content from 10.5 to 16.5%. Minimum and maximum mean values for the glutens from different flours were 40 and 85 g for resistance, and 28 and 36 cm for extensibility, and differences between flours were highly significant. Resistance and extensibility were inversely correlated ($r = -.95$; 1% = .83), but neither was correlated with the protein content or loaf volume of the flour. A differential behavior of glutens from different samples to washing was established; regressions for resistance (but not for extensibility) on gluten protein differed significantly.

Glutens from composite samples of six varieties showed no significant differences in extensibility, but mean resistance values ranged from 60 to 88 g and differed significantly. No significant relation was found between gluten properties and protein content or loaf volume of the flours. It was noted that the varieties were placed in the same order for decreasing resistance as for decreasing general strength of doughs as measured by the farinograph and the mixograph.

Acknowledgment

The authors are indebted to Mr. H. E. Rasmussen for his help in the construction of the Stretchometer.

Literature Cited

Baker, J. C., Mize, M. D., and Parker, H. K.
1943 Measurement and significance of gluten quality. Cereal Chem. **20:** 506–516.
——, Parker, H. K., and Mize, M. D.
1942 The action of an oxidizing agent in bread dough made from patent flours. Cereal Chem. **19:** 334–346.
Bailey, C. H.
1940 Physical tests of flour quality. Wheat Studies of the Food Research Inst. **16:** 243–300.
Fisher, E. A., and Halton, P.
1936 Observations on gluten washing. Cereal Chem. **13:** 575–591.
Geddes, W. F., Aitken, T. R., and Fisher, M. H.
1940 The relation between the normal farinograms and the baking strength of Western Canadian wheat. Cereal Chem. **17:** 528–551.
James, T. R., and Huber, L. X.
1927 A study of the physical properties of washed gluten. Cereal Chem. **4:** 449–460.
Kress, C. B.
1924 Gluten quality. Cereal Chem. **1:** 247–250.
Mohs, K., Schmidt, E. A., and Frank, H. V.
1939 Die Bestimmung der Kleberdehnberkeit mittels des Glutographen. Z. ges. Getreide. u. Mühlenw. **26:** 23–30.

ESSENTIAL AMINO ACID COMPOSITION OF FEED MATERIALS

WERNER BAUMGARTEN,[1] ADALINE N. MATHER, and LEONARD STONE

Hiram Walker & Sons, Inc., Peoria, Illinois

(Received for publication August 10, 1945)

A large number of feed materials have been analyzed for 12 amino acids by microbiological assay. Three microorganisms, *Lactobacillus arabinosus*, *Lactobacillus casei*, and *Streptococcus faecalis* R, have been employed in these determinations. *L. arabinosus* and *L. casei* have been used extensively and to a high degree of satisfaction in microbiological amino acid determinations. Assays using *S. faecalis* R were previously reported, but as details were not given they shall be discussed in this report.

An improvement of our earlier procedure for *S. faecalis* R was introduced by using acid production instead of turbidity as measurement of growth. The medium of Teply and Elvehjem (1945) for folic acid with *S. faecalis* R was modified for amino acid determinations. Sodium citrate was added to our *S. faecalis* R medium as an additional buffer. Higher acidity was produced with such a medium, which allowed evaluation of the assay by titration of the acid formed, but sodium citrate intensified caramelization of the glucose during autoclaving and consequently the detection of the indicator end point was hindered. This difficulty was overcome by sterilizing the medium separately by passing it through a Seitz filter. The medium was inoculated and added aseptically to the autoclaved culture tubes containing the hydrolyzates. This adaptation was found satisfactory in assays with *S. faecalis* R, provided pyridoxamine was added, except in assays for lysine and tyrosine. Their outstanding behavior will be referred to below.

The techniques establishing the reliability of the assays and their application to feed materials are described. The analyzed materials are appraised in terms of essential amino acids and their nutritional significance has been discussed.

Experimental Technique

Microbiological Procedure. Three microorganisms, *Lactobacillus arabinosus*, *Lactobacillus casei*, and *Streptococcus faecalis* R (formerly known as *Streptococcus lactis* R), were employed. Stock cultures were carried in neutral tomato juice agar, containing 400 ml clarified tomato

---

[1] Present address, J. T. Baker Chemical Co., Phillipsburg, N. J.

TABLE I

COMPOSITION OF MEDIUM[1] FOR MICROBIOLOGICAL ASSAY OF AMINO ACIDS[2] WITH *Streptococcus faecalis* R

Components	Medium I[3] For turbidity measurement (medium autoclaved)	Medium II For titration measurement (medium autoclaved)	Medium III For titration measurement (medium sterilized by filtration)
Glucose	100.0 mg	100.0 mg	100.0 mg
Sodium acetate anhyd.	60.0 mg	60.0 mg	60.0 mg
Sodium citrate anhyd.	—	200.0 mg	200.0 mg
Adenine	0.5 mg	0.5 mg	0.5 mg
Guanine	0.5 mg	0.5 mg	0.5 mg
Uracil	0.5 mg	0.5 mg	0.5 mg
Xanthine	0.5 mg	0.5 mg	0.5 mg
K_2HPO_4	5.0 mg	5.0 mg	5.0 mg
KH_2PO_4	5.0 mg	5.0 mg	5.0 mg
$MgSO_4 \cdot 7\ H_2O$	2.0 mg	2.0 mg	2.0 mg
$MNSO_4 \cdot 4\ H_2O$	0.1 mg	0.1 mg	0.1 mg
NaCl	0.1 mg	0.1 mg	0.1 mg
$FeSO_4 \cdot 7\ H_2O$	0.1 mg	0.1 mg	0.1 mg
Thiamine hydrochloride	10.0 γ	10.0 γ	10.0 γ
Pyridoxine hydrochloride	2.0 γ	2.0 γ	
Calcium pantothenate	2.0 γ	2.0 γ	2.0 γ
Riboflavin	2.0 γ	2.0 γ	2.0 γ
Nicotinic acid	4.0 γ	4.0 γ	4.0 γ
p-Aminobenzoic acid	2.0 γ	2.0 γ	2.0 γ
Biotin	0.05 γ	0.05 γ	0.05 γ
Pyridoxamine hydrochloride			10.0 γ
Folic acid[4]	2.5 mg units	2.5 mg units	2.5 mg units
dl Alanine	4.0 mg		
l(+) Arginine hydrochloride	2.0 mg		
Asparagine	4.0 mg		
l(−) Cystine	2.0 mg		
l(+) Glutamic acid	4.0 mg		
Glycine	2.0 mg		
l(−) Histidine hydrochloride	2.0 mg		
l(−) Hydroxyproline	2.0 mg		
dl Isoleucine	2.0 mg		
l(−) Leucine	2.0 mg		
dl Lysine hydrochloride	2.0 mg		
dl Methionine	2.0 mg		
dl Norleucine	2.0 mg		
dl Phenylalanine	2.0 mg		
l(−) Proline	2.0 mg		
dl Serine	2.0 mg		
dl Threonine	2.0 mg		
l(−) Tryptophane	2.0 mg		
l(−) Tyrosine	2.0 mg		
dl Valine	2.0 mg		

---

[1] The medium was adjusted to pH 6.8–7.0 and diluted to contain the contents of one assay tube in 5 ml of solution.

[2] The amino acid to be assayed was omitted from the medium.

[3] The medium in column I was used in assays with *L. arabinosus* and *L. casei*.

[4] Vitamin B_c was employed in assays for tryptophane as the impure liver eluate contains some tryptophane. Norit-treated casein hydrolyzate was used in these assays (Greene and Black, 1944). One mg unit of folic acid is defined as the activity of 1 mg of a standard preparation of solubilized liver powder (Wilson & Co.). University of Texas publication No. 4137, p. 36, "Assay Method for Folic Acid," Mitchell and Snell.

juice, 10 g yeast extract, 10 g glucose, 5 ml each of salt A and B (Williams, 1941), and 15 g agar in one liter. The inoculum was prepared in tomato juice broth of pH 5.5 containing 400 ml tomato juice, 10 g Neopeptone, 10 g bactopeptonized milk, 10 g yeast extract, 10 g glucose, 5 ml each of salt A and B, and 2 ml of liver eluate prepared according to Hutchings, Bohonos, and Peterson (1941) in one liter of solution and incubated at 37°C for 18 hours before use.

The assay technique for *L. arabinosus* and *L. casei* has been described (Baumgarten, Garey, Olsen, Stone, and Boruff, 1944; and Baumgarten, Stone, and Boruff, 1945).

S. faecalis R has been employed (see Snell and Guirard, 1943, for preliminary report on its amino acid requirements) in microbiological assays of grain alcohol fermentation by-products (Baumgarten, Stone, and Boruff, 1945). In those earlier assays growth was measured by turbidity. The composition of the media for *S. faecalis* R is given in Table I. The medium in column I (it should be noted that this is the medium employed in assays with *L. arabinosus* and *L. casei*) was adequate in assays in which turbidity was considered a satisfactory measurement. The addition of 2% sodium citrate (medium, column II) buffers the medium sufficiently to allow production of the maximal amount of lactic acid. The dipotassium phosphate of Teply and Elvehjem's folic acid medium was omitted as no advantage was observed, and instead 0.6% sodium acetate was added. The addition of sodium citrate intensified the caramelization and dark color of the final bacterial suspension, and impeded titration against a color indicator.

This difficulty was overcome by sterilizing the medium and the samples separately. Suitable aliquots of the unknown neutral hydrolyzates or the neutralized solutions of the amino acid for establishing the standard curve were introduced into the assay tubes (25 × 200 mm) and the volume adjusted to 5 ml with distilled water. The tubes were plugged with cotton and autoclaved. The medium was sterilized separately by passing through a Seitz filter and then inoculated with the bacterial suspension. Five ml of the inoculated medium were added aseptically to the autoclaved tubes containing the amino acid solutions or the hydrolyzates. It was observed, however, that acid production was only 80% with such a medium as compared to the acid formed with the same medium sterilized by autoclaving. It was established that this was due to a pyridoxamine deficiency and that the medium (column III) containing pyridoxamine supported maximal growth and gave identical results regardless of the method of sterilization. An explanation of this phenomenon was indicated by Snell and Rannefeld (1945) who showed that pyridoxamine is the active vitamin for *S. faecalis* R and not pyridoxine itself. Snell (1945a) proved that pyridoxamine was formed during heating of pyridoxine with amino acid solutions, and established (1945) the natural occurrence of pyridoxamine. From our experiments it is apparent that the addition of

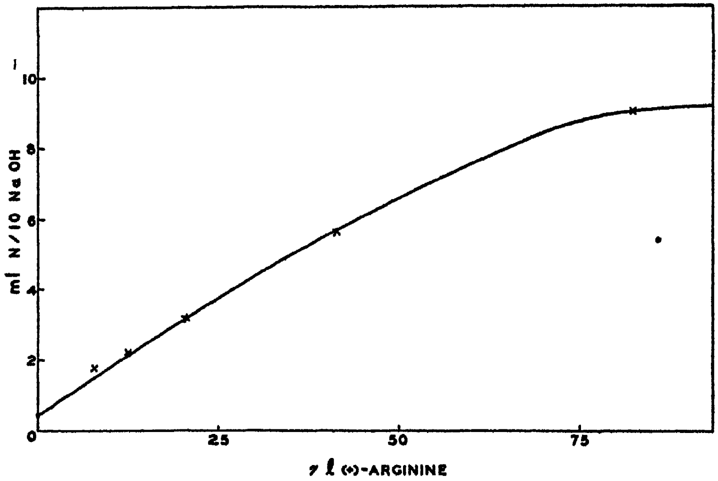


Fig. 1. Standard curve for arginine with *S. faecalis* R, employing medium III.

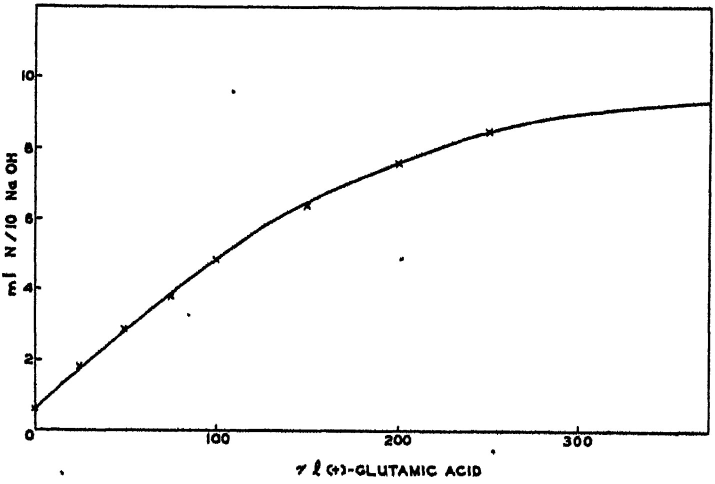


Fig. 2. Standard curve for glutamic acid with *S. faecalis* R, employing medium III.

pyridoxamine was essential if the medium was not heat sterilized. It must be assumed that suboptimal amounts of pyridoxamine were formed at 30°C by the interaction of pyridoxine and amino acid solutions. Assay results tended to be higher when the filter-sterilized medium devoid of pyridoxamine was employed. More titratable acid was formed in the tubes containing the natural hydrolyzates than in those tubes used to establish the standard curve when compared in terms of assayed amino acid present. This was due to the fact that pyridoxamine was present in the hydrolyzates and led to a wrong interpretation of the results. Again, the necessity for nutritionally complete media must be pointed out. The role of glutamic acid (Snell, 1945a) in the transamination reaction was strongly indicated

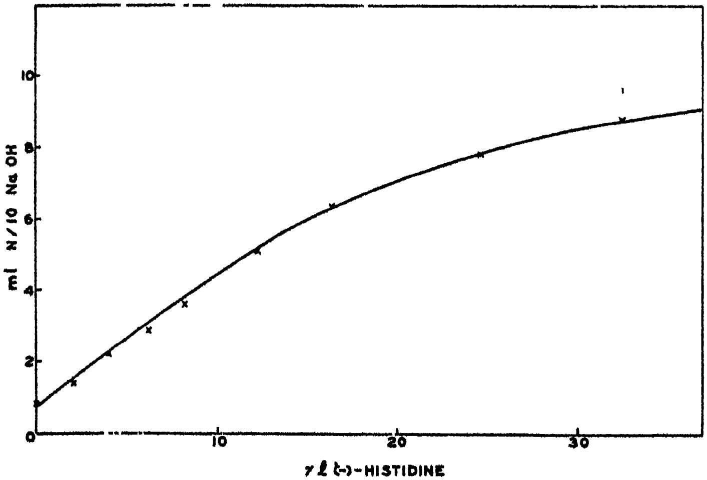


Fig. 3. Standard curve for histidine with *S. faecalis* R, employing medium III.

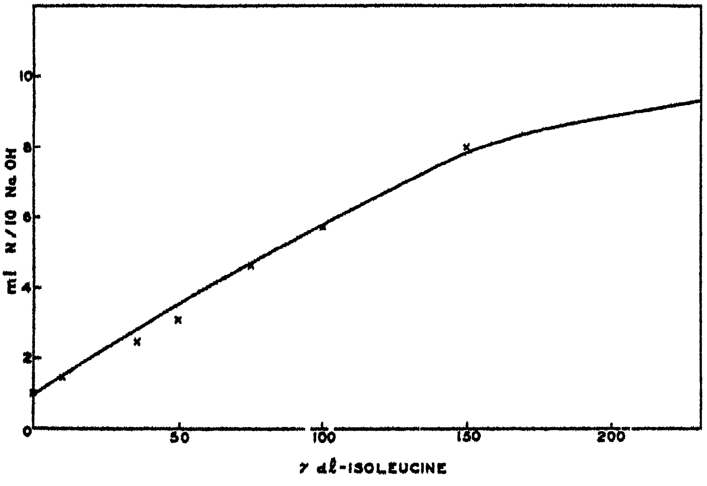


Fig. 4. Standard curve for isoleucine with *S. faecalis* R, employing medium III.

as its assays showed the greatest discrepancies in the absence of pyridoxamine.

The medium in column III (Table I) was adopted for assays of arginine, glutamic acid, histidine, isoleucine, leucine, methionine, serine, threonine, tryptophane, and valine with *S. faecalis* R. Standard curves for these amino acids are presented in Figures 1 to 5, 7 to 10, and 12. Lysine (Figure 6) and tyrosine (Figure 11) were determined employing the medium in column II.

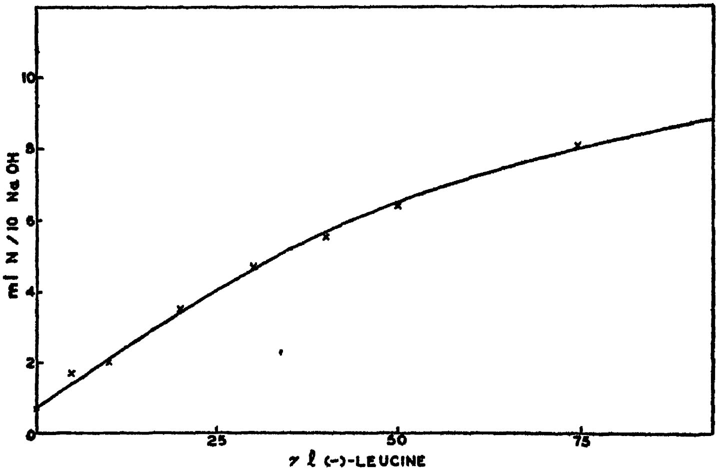


Fig. 5. Standard curve for leucine with *S. faecalis* R, employing medium III.

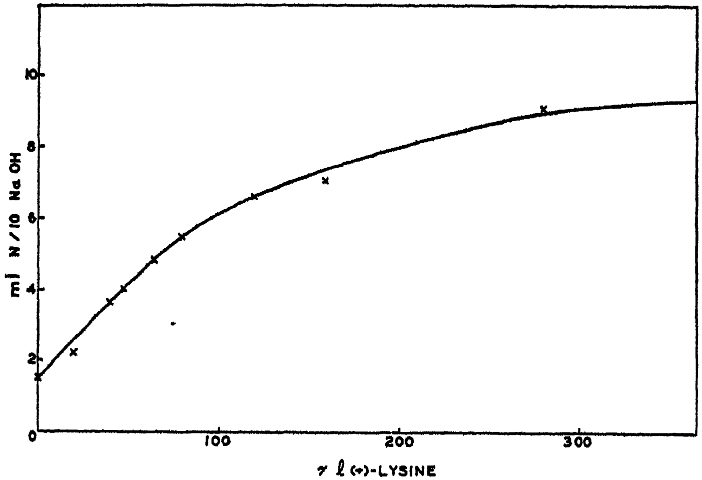


Fig. 6. Standard curve for lysine with *S. faecalis* R, employing medium II.

The essential character of tyrosine was dependent on the amount of pyridoxamine present in the medium. The amount of pyridoxamine formed during autoclaving of the pyridoxine with the amino acid solutions or hydrolyzates did not interfere, however, the addition of 1 μg of pyridoxamine per assay tube resulted in near maximal blank titration. Results obtained with the medium given in column II were satisfactory and in close agreement with values determined with *L. casei*. Experiments showed that pyridoxal, the pyridoxine analogue

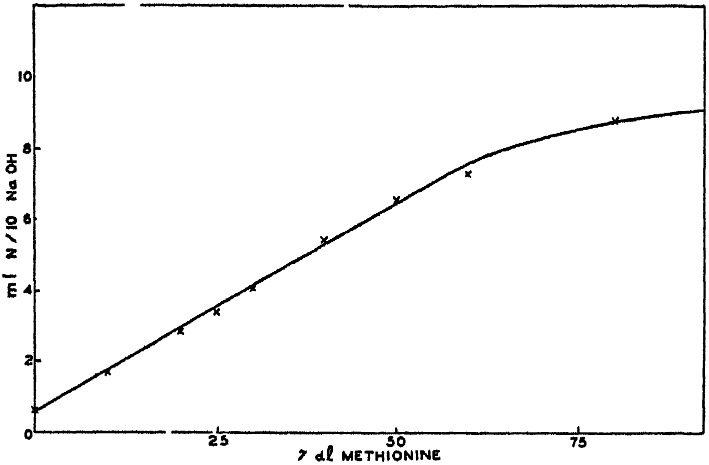


Fig. 7. Standard curve for methionine with *S. faecalis* R employing medium III

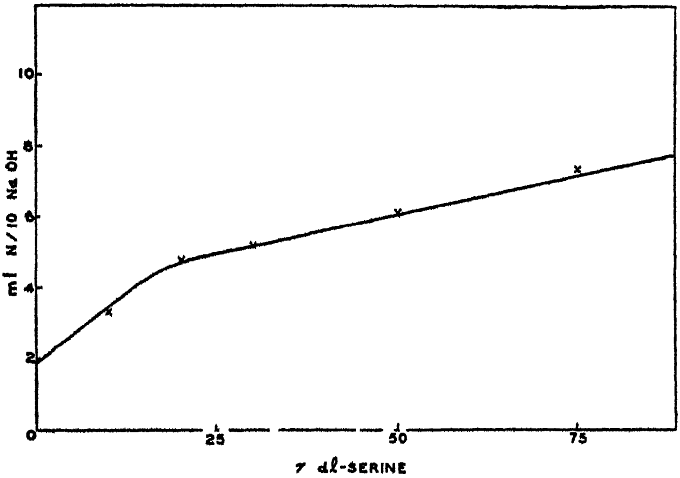


Fig. 8. Standard curve for serine with *S. faecalis* R employing medium III

essential for the growth of *L. casei*, did not interfere with tyrosine determinations employing *L. casei*.

Lysine exhibited an unexplained behavior. Standard curves of lysine with *S. faecalis* R established with medium III showed a higher blank titration than the next tubes, resulting in a dip in the curve.

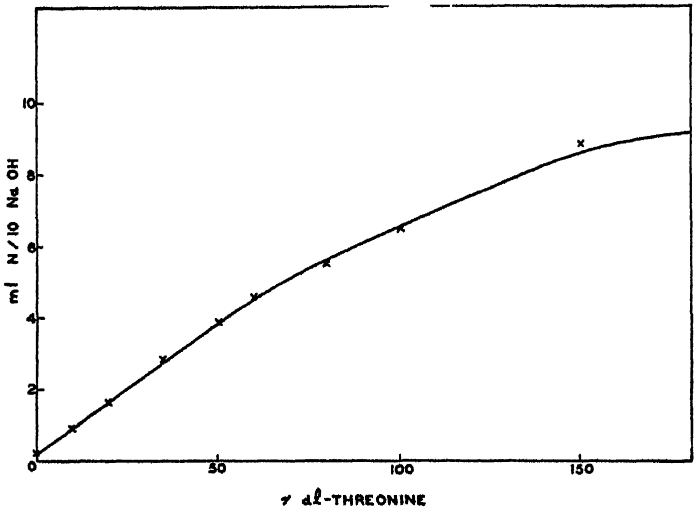


Fig. 9. Standard curve for threonine with *S. faecalis* R, employing medium III.

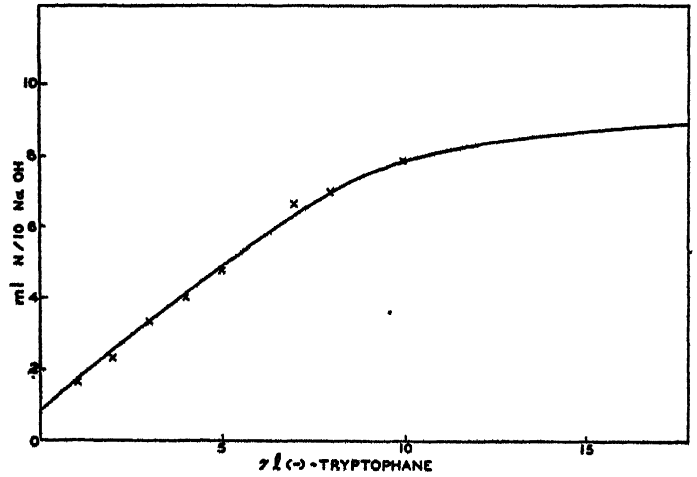


Fig. 10. Standard curve for tryptophane with *S. faecalis* R, employing medium III.

The medium in column II was found satisfactory for both lysine and tyrosine and was adopted for their determinations.

Reliability of Assays with S. faecalis R Smooth standard curves, recovery of added amino acid within the limit of experimental error, and identical results at various levels of concentration of amino acid were established in assays of amino acid with *S. faecalis* R. Recovery figures for methionine and serine are included in this report as they were not reported previously. These recovery experiments were carried out by addition of amino acid to the material. From these

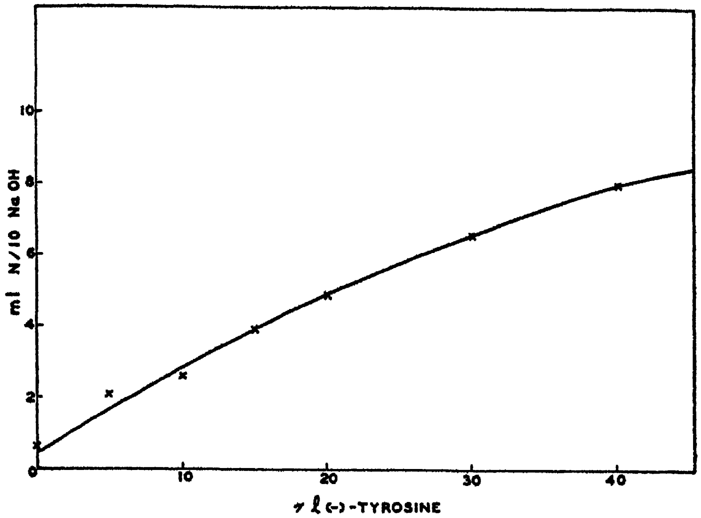


Fig. 11 Standard curve for tyrosine with *S faecalis* R employing medium II

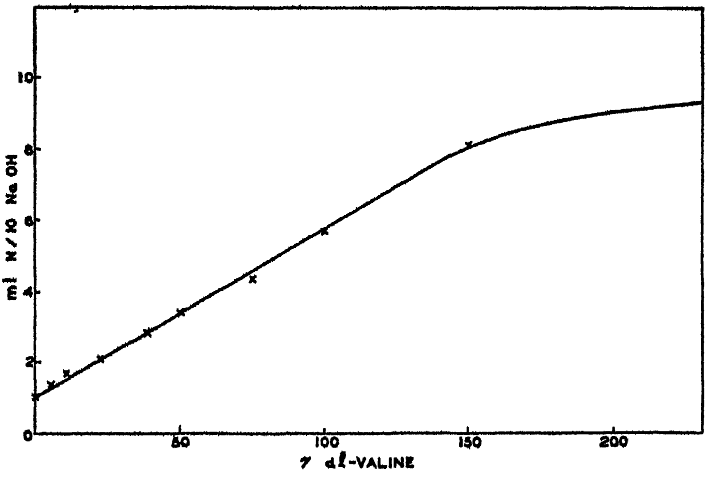


Fig 12 Standard curve for valine with *S faecalis* R, employing medium III

results it can be observed that recovery is well within the limits of the experimental error, a fact which invalidates claims that growth inhibitors might be present in such hydrolyzates (Table II).

TABLE II

RECOVERY DATA ON METHIONINE AND SERINE IN ASSAYS WITH *S. faecalis* R

Material investigated	Per cent found		Per cent recovery
	Methionine	Serine	
Corn	0.15	—	—
Corn + 0.25% *l*(–) methionine	0.38	—	92
Wheat	0.15	0.56	—
Wheat + 0.25% *l*(–) methionine	0.38	—	92
Wheat + 1.25% *l*(–) serine	—	1.7	91
Corn solubles	—	1.2	—
Corn solubles + 1.25% *l*(–) serine	—	2.5	104
Tankage	—	2.2	—
Tankage + 1.25% *l*(–) serine	—	3.4	96

In addition two commercial purified proteins were analyzed with five microorganisms for the purpose of comparing the results and proving their reliability. Twelve amino acids were determined in casein and gelatin and the results compiled in Tables III and IV. In most instances the values agreed very closely. The results for glutamic acid were discussed previously (Baumgarten, Mather, and Stone,

TABLE III

COMPARISON OF AMINO ACID COMPOSITION OF CASEIN [1] AS DETERMINED MICROBIOLOGICALLY WITH FIVE MICROORGANISMS

	Optical form of amino acid used for standard curve	Amino acid composition (dry matter basis) as determined by [2]					Chemical [3] analysis
		L. acidophilus	*L. arabinosus*	*L. casei*	*L. mesenteroides*	*S. faecalis* R	
		%	%	%	%	%	%
Arginine	*l*(+)	4.1	—	3.6	4.0	3.8	3.8
Histidine	*l*(–)	3.7	—	—	3.2	3.3	2.3
Isoleucine	*dl*	7.3	7.7	7.8	7.6	7.5	6.0
Leucine	*l*(–)	10.8	10.7	11.2	11.0	9.6	11.3
Lysine	*l*(+)	—	—	—	—	8.3	6.4
Methionine	*dl*	3.0	2.7	—	3.1	3.0	3.2
Phenylalanine	*dl*	4.9	—	5.0	—	—	4.9
Serine	*dl*	—	—	—	—	6.3	6.0
Threonine	*dl*	—	4.5	—	—	4.1	3.6
Tryptophane	*l*(–)	0.9	1.0	0.9	—	0.9	1.6
Tyrosine	*l*(–)	5.0	—	5.5	—	5.0	5.9
Valine	*dl*	—	7.4	6.9	8.1	7.5	6.5

[1] Labco casein: 3.7% moisture, 14.84% nitrogen (on dry basis).
[2] For description of organisms and medium employed see Baumgarten, Mather, and Stone (1945).
[3] Block and Bolling (1945).

TABLE IV

COMPARISON OF AMINO ACID COMPOSITION OF GELATIN[1] AS DETERMINED MICROBIOLOGICALLY WITH FIVE MICROORGANISMS

	Optical form of amino acid used for standard curve	Amino acid composition (dry matter basis) as determined by[2]					Chemical[3] analysis
		L. acido-philus	*L. arabi-nosus*	*L. casei*	*L. mesen-teroides*	*S. faecalis* R	
		%	%	%	%	%	%
Arginine	*l*(+)	8.2	—	7.9	8.4	8.3	8.2
Histidine	*l*(−)	1.0	—	0.8	0.8	1.0	1.1
Isoleucine	*dl*	1.9	1.9	2.3	1.9	1.9	1.8
Leucine	*l*(−)	3.6	3.7	3.6	3.6	3.2	4.0
Lysine	*l*(+)	—	—	—	—	5.1	4.7
Methionine	*dl*	1.0	0.9	—	0.9	1.0	0.9
Phenylalanine	*dl*	2.3	—	2.1	—	—	2.2
Serine	*dl*	—	—	—	—	4.0	3.6
Threonine	*dl*	—	2.0	—	—	1.9	1.6
Tyrosine	*l*(−)	0.8	—	0.7	—	0.6	0.2
Valine	*dl*	—	3.1	2.5	3.7	2.7	2.7

[1] Difco gelatin: 10.8% moisture, 17.23% nitrogen (dry basis).
[2] For description of organisms and medium employed see Baumgarten, Mather, and Stone (1945).
[3] Block and Bolling (1945).

TABLE V

COMPARISON OF AMINO ACID VALUES EMPLOYING THE DIFFERENT MEDIA[1] IN ASSAYS WITH *S. faecalis* R

Amino acid	Medium employed	Corn	Wheat	Corn solubles	Wheat-Milo grains with solubles	Milo grains with solubles
Arginine	I	—	—	—	1.0	0.78
	III	—	—	—	1.0	0.80
Histidine	I	-	0.39	--	0.64	0.76
	II	-	—	—	0.61	0.62
	III		0.28	--	0.58	0.65
Glutamic acid	I	2.0	3.9		-	- -
	III	1.96	3.6	-	—	—
Lysine	I	0.27	0.38	—	—	-
	II	0.28	0.38	—	—	—
Serine	II	-	0.56	1.15	—	—.
	III	—	0.64	1.22	—	—
Threonine	I	—	—	—	0.96	1.0
	III	—	—	—	0.89	0.96
Tyrosine	I	—	0.12	—	—	0.75
	II	—	0.10	—	—	0.77

[1] The media are described in Table I, columns I, II, and III.

1945). For comparison, values determined chemically were included (Block and Bolling, 1945).

Amino acid values determined with *S. faecalis* R were independent regardless of the medium (Table I) employed. Comparisons were carried out for several amino acids employing the different media and the results are given in Table V. For isoleucine, leucine, methionine, tryptophane, and valine, results obtained with medium III with *S. faecalis* R were compared with those determined with *L. arabinosus*. These data are given in Table VI. These compilations might appear superfluous, but similar results obtained on natural hydrolyzates strengthen the degree of reliability which can be assigned to such results, and they give additional information with respect to the possible occurrence of growth inhibitors.

TABLE VI

COMPARISON OF ASSAY RESULTS OF SOME NATURAL PRODUCTS DETERMINED WITH *S. faecalis* R AND *L. arabinosus*[1]

Amino acid	Determined with	Corn	Wheat	Fish solubles	Red Dog flour	Milo-wheat solubles	Milo-wheat grains with solubles
Isoleucine	*S. faecalis* R	0.36	0.50	—	—	—	—
	L. arabinosus	0.44	0.57	—	—	—	—
Leucine	*S. faecalis* R	1.16	0.86	—	—	—	—
	L. arabinosus	1.37	0.91	—	—	—	—
Methionine	*S. faecalis* R	0.19	0.17	—	—	0.40	—
	L. arabinosus	0.18	0.14	—	—	0.36	—
Tryptophane	*S. faecalis* R	—	—	0.30	0.17	—	—
	L. arabinosus	—	—	0.30	0.16	—	—
Valine	*S. faecalis* R	0.40	—	—	—	—	1.5
	L. arabinosus	0.46	—	—	—	—	1.7

[1] Medium III was employed with *S. faecalis* R; medium I with *L. arabinosus*.

Specificity of Response. On first assumption only the naturally occurring *l* amino acids were supposed to possess growth-promoting activity (Stokes and Gunness, 1944). The substitution of optical antipodes or closely related compounds of specific amino acids for *L. arabinosus*, *L. casei*, and *S. faecalis* R has been investigated. Only in the case of glutamic acid (Baumgarten, Mather, and Stone, 1945) has the *d* form been found to be partially active. Under our experimental conditions *dl* leucine possessed half the activity of *l*(−) leucine and *d*(+) leucine was entirely ineffective to promote growth of any of the three organisms (Table VII). Hegsted (1945) reported *d*(+) leucine

TABLE VII

Response of *L. arabinosus*, *L. casei*, and *S. faecalis* R to the Different Optical Forms of Leucine

Leucine added per tube of 10 ml			Growth produced by [1]		
l(−)	*dl*	*d*(+)	*L. arabinosus*	*L. casei*	*S. faecalis* R
γ	γ	γ	ml *N*/10 NaOH	ml *N*/10 NaOH	ml *N*/10 NaOH
0	0	0	0.9	0.8	0.6
25	0	0	4.15	3.9	3.7
0	50	0	4.0	3.75	3.5
50	0	0	6.55	6.4	6.05
0	100	0	6.45	6.5	5.7
0	0	100	0.9	0.9	0.7
50	0	21,000	—	—	7.15
100	0	0	8.5	8.1	—
100	0	21,000	9.0	8.8	—

[1] Medium I was employed with *L. arabinosus* and *L. casei*, medium III with *S. faecalis* R. The media were complete, only leucine was omitted.

TABLE VIII

Response of the Microorganisms to the *l* and *dl* Forms of Lysine, Methionine, Tryptophane, and Valine

Medium contained per tube		Growth produced by [1]		
l	*dl*	*L. arabinosus*	*L. casei*	*S. faecalis* R
γ	γ	ml *N*/10 NaOH	ml *N*/10 NaOH	ml *N*/10 NaOH
Lysine				
0	0	—	—	1.6
40	0	—	—	3.0
0	80	—	—	2.9
100	0	—		4.65
0	200	—		4.55
Methionine				
0	0	—	—	0.65
10	0	—	—	2.7
0	20	—	—	3.0
35	0		—	7.6
0	70		—	8.1
Tryptophane				
0	0	1.9	—	0.0
1	0	3.0	—	1.65
0	2	3.15	—	1.7
2	0	4.15	—	2.3
0	4	4.3		2.5
3	0	4.8	—	3.35
0	6	5.1	—	3.5
Valine				
0	0	0.8	1.3	82.2
10	0	2.45	2.8	63.75
0	20	2.55	2.7	61.5
20	0	3.7	3.6	56
0	40	3.9	3.6	53
50	0	7.0	5.55	35
0	100	7.2	5.3	34

[1] Medium I was used with all three organisms, in the case of valine. In the determinations of lysine, methionine, and tryptophane, medium III was employed with *S. faecalis* R. The figures with *S. faecalis* R for valine are turbidity readings on a arbitrary scale.

active for *L. arabinosus*. Neither was an inhibition of growth observed when *d*(+) leucine was added to assay tubes containing *l*(−) leucine under our experimental conditions, contrary to reports of Fox, Fling, and Bollenback (1944) for *L. arabinosus*.

S. faecalis R required twice the amount of *dl* lysine to produce the same quantity of acid produced from *l*(+) lysine. Similar results were observed with methionine and tryptophane for *L. arabinosus* and *S. faecalis* R and with valine for *L. arabinosus*, *L. casei*, and *S. faecalis* R (Table VIII).

A number of compounds derived from the corresponding amino acids were tested for growth-promoting activity (Table IX). Histamine did not replace histidine, nor did cadaverine replace lysine for *S. faecalis* R. Indole and anthranilic acid were reported to be stimulatory for *L. arabinosus* (Greene and Black, 1943). Employing the present experimental technique, indole replaced tryptophane for *L. arabinosus*, but was entirely inactive in replacing tryptophane for *S. faecalis* R. In high concentrations it inhibited growth for both organisms. Anthranilic acid substituted for tryptophane only for *L. arabinosus* but not for *S. faecalis* R. No inhibitory activity was exhibited. Tryptamine and indole-3-acetic acid were ineffective.

TABLE IX

RESPONSE OF *L. arabinosus* AND *S. faecalis* R TO COMPOUNDS RELATED TO GROWTH-PROMOTING AMINO ACIDS IN MEDIA DEVOID OF THE AMINO ACID

Medium contained	Growth produced by [1]	
	L. arabinosus ml *N*/10 NaOH	*S. faecalis* R ml *N*/10 NaOH
0 *l*(−) histidine	—	0.8
0 *l*(−) histidine + 500 γ histamine	—	1.0
160 γ *l*(−) histidine	—	9.2
160 γ *l*(−) histidine + 2000 γ histamine	—	9.2
0 *l*(+) lysine	—	1.5
0 *l*(+) lysine + 500 γ cadaverine	—	2.3
80 γ *l*(+) lysine	—	5.5
80 γ *l*(+) lysine + 4000 γ cadaverine	—	5.4
0 *l*(−) tryptophane	0.65	0.0
0 *l*(−) tryptophane + 100 γ indole	6.8	0.0
0 *l*(−) tryptophane + 100 γ anthranilic acid	5.4	0.0
0 *l*(−) tryptophane + 500 γ tryptamine	1.4	0.0
0 *l*(−) tryptophane + 1000 γ indole-3-acetic acid	1.8	0.0
10 γ *l*(−) tryptophane	6.6	5.3
10 γ *l*(−) tryptophane + 4000 γ indole	0.65	0.0
10 γ *l*(−) tryptophane + 4000 γ anthranilic acid	6.6	4.3
10 γ *l*(−) tryptophane + 4000 γ tryptamine	6.3	4.2
10 γ *l*(−) tryptophane + 4000 γ indole-3-acetic acid	6.3	5.7

[1] Medium I was employed with *L. arabinosus*; medium III with *S. faecalis* R.

Recently Stokes and Gunness (1945) demonstrated that only the *l* form of serine promoted growth for *L. delbruckii.*

Preparation and Assay of Samples. Preparation of the samples for tryptophane determination was accomplished by autoclaving with saturated barium hydroxide solution for 7 hours at 15 pounds pressure (Greene and Black, 1944). Refluxing for 20 hours with 20% hydrochloric acid was employed in processing the materials for the assay of the other amino acids. Stability of the individual amino acids under the conditions of hydrolysis was investigated by adding the amino acids one by one to some samples and determining the percentage of recovery (see Baumgarten, Stone, and Boruff, 1945).

Arginine, phenylalanine, and tyrosine were determined with *L. casei;* isoleucine, leucine, methionine, tryptophane, and valine with *L. arabinosus;* and arginine, histidine, isoleucine, leucine, lysine, methionine, serine, threonine, tryptophane, tyrosine, and valine with *S. faecalis* R. The glutamic acid values were included for comparison and were discussed previously (Baumgarten, Mather, and Stone, 1945).

The tryptophane determinations were carried out using norit-treated casein hydrolyzate supplemented with cystine as the source of amino acids (Greene and Black, 1944). In these assays crystalline vitamin B_6 was substituted for liver eluate as the latter nearly always contained some tryptophane. In the calculation of the tryptophane content of the samples, the observed values were multiplied by two since the tryptophane is completely racemized by alkaline hydrolysis.

Discussion of Results

The analytical data are compiled in Table X. The results are expressed in the percentage of amino acid contained in the dried materials. The nitrogen content of the samples is given, and the nitrogen percentage accounted for by the amino acids has been calculated.

Relatively little data are available in the literature on the amino acid composition of complex materials such as the feeds investigated. A very excellent review covering the literature up to 1943 has been compiled by Block and Bolling (1945). We shall not attempt to compare individual values, as samples of this nature vary considerably, depending on their source and origin. It might be sufficient to note that for the materials contained in Block and Bolling's compilation the values given are of the same order of magnitude as ours, with the exception of leucine and isoleucine. The leucine values are higher and the isoleucine values lower than our results, which might be attributed to the lack of dependability of the chemical method for the two amino acids.

TABLE X

AMINO ACID COMPOSITION OF FEED

(Dry matter basis)

	Nitrogen	Arginine	Glutamic acid [1]	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Serine	Threonine	Tryptophane	Tyrosine	Valine	Per cent nitrogen accounted for by amino acids in dry material
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Grains & grain by-products															
Wheat	2.6	0.5	3.9	0.4	0.7	1.0	0.4	0.2	0.7	0.7	0.4	0.2	0.1	0.7	47.28
Granular wheat flour	2.4	0.4	3.8	0.3	0.7	1.0	0.3	0.1	0.7	0.6	0.3	0.1	0.2	0.6	46.01
Corn	1.5	0.4	2.1	0.3	0.6	1.4	0.3	0.2	0.5	0.8	0.3	0.05	0.2	0.5	62.66
Rye	2.9	0.6	3.6	0.4	0.7	1.0	0.6	0.1	0.7	0.7	0.4	0.1	0.2	0.7	42.48
Milo maize	1.9	0.4	2.6	0.3	0.7	1.8	0.3	0.1	0.6	0.6	0.3	0.1	0.2	0.7	44.35
Ground barley	2.9	0.5	3.2	0.3	0.7	1.0	0.4	0.2	0.7	0.5	0.4	0.2	0.2	0.7	39.59
Barley malt	2.4	0.5	2.5	0.3	0.7	0.9	0.6	0.2	0.6	0.5	0.4	0.2	0.2	0.7	45.58
Gluten feed	4.6	0.7	4.5	0.8	1.3	3.0	0.8	0.4	1.0	0.9	1.0	0.1	0.4	1.5	44.41
Gluten meal	7.1	1.2	9.4	1.0	2.4	7.3	0.8	0.9	2.5	1.7	1.4	0.2	1.1	2.1	52.76 [2]
Wheat bran	3.0	0.9	—	0.5	0.8	1.1	0.6	0.2	0.6	1.0	0.4	0.3	0.2	0.9	37.88
Oats, finely ground	2.7	0.7	2.9	0.4	0.7	1.1	0.6	0.2	0.8	0.4	0.4	0.1	0.2	0.7	45.36
Standard wheat middlings	3.7	1.3	4.5	0.6	1.1	1.3	0.6	0.2	1.0	0.9	0.6	0.2	0.3	1.2	51.16
Red Dog flour	3.8	0.9	—	0.6	1.0	1.3	0.7	0.2	0.5	1.1	0.6	0.2	0.2	1.2	34.76 [2]
Distillers' by-products															
Corn grains with solubles	5.1	0.9	5.7	0.8	2.0	2.4	0.7	0.5	2.1	1.9	1.1	0.1	0.7	1.8	49.77
Corn solubles	4.4	0.6	4.3	0.7	1.5	1.4	0.7	0.4	1.7	1.2	0.9	0.1	0.6	1.4	43.80
Rye grain with solubles	5.1	1.2	5.5	0.7	1.6	2.2	1.1	0.4	1.4	1.3	1.1	0.3	0.5	1.7	47.16
Rye solubles	6.4	1.4	—	0.8	2.0	1.9	0.7	0.5	1.8	1.7	1.2	0.2	0.6	2.1	33.18 [2]
Wheat grains with solubles	5.4	1.4	9.3	0.8	2.0	2.6	1.0	0.5	1.7	1.8	1.2	0.3	0.6	2.1	57.08
Milo grains with solubles	5.8	1.1	7.6	1.0	0.9	1.8	0.9	0.5	1.9	2.2	1.1	0.3	0.9	2.1	48.10
Wheat-Milo grains with solubles	5.1	1.2	7.7	0.8	1.8	3.5	1.0	0.5	1.8	1.7	1.0	0.3	0.8	1.9	57.96
Wheat-Milo solubles	4.9	1.2	7.9	0.7	1.6	1.9	0.8	0.4	1.4	2.1	1.1	0.2	0.6	1.5	53.78
Butyl alcohol fermentation by-products	4.9	0.7	—	0.5	1.8	2.2	1.2	0.4	1.0	1.0	0.5	0.2	0.5	1.7	32.59 [2]

TABLE X—*Continued*

	Nitrogen	Arginine	Glutamic acid[1]	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Serine	Threonine	Tryptophane	Tyrosine	Valine	Per cent nitrogen accounted for by amino acids in dry material
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Vegetable protein concentrates															
Cottonseed meal	6.4	3.0	6.8	1.1	1.8	2.2	1.5	0.5	2.2	1.8	1.1	0.4	0.6	1.8	53.22
Extracted soybean meal	9.1	3.0	9.0	1.7	3.5	4.5	3.0	0.9	2.8	2.4	2.1	0.6	1.2	3.0	56.00
Expelled soybean meal	7.6	2.8	7.8	1.2	2.9	4.0	3.2	0.4	2.2	2.2	2.2	0.5	0.9	2.6	59.63
Animal by-products															
Tankage	11.3	4.3	7.9	1.9	2.8	6.6	3.5	0.6	3.1	2.3	2.4	0.5	1.5	4.0	51.12
Meat scraps	10.0	3.4	6.4	2.2	2.1	5.0	3.8	0.7	3.2	2.3	2.2	0.4	1.4	3.8	52.28
Liver meal	13.0	4.0	8.1	1.8	3.7	6.7	4.9	1.2	3.9	2.7	2.6	0.5	1.8	4.2	48.85
Liver powder	10.0	1.7	8.3	1.1	1.3	1.8	4.2	0.4	0.7	2.2	1.5	0.2	0.6	1.2	34.85
Fish meal	12.0	4.5	9.2	2.3	4.9	6.4	6.6	1.8	3.5	2.2	3.1	0.6	2.3	4.5	60.96
Fish solubles	11.1	2.6	—	5.0	1.6	1.8	2.3	0.8	1.2	2.2	1.2	0.3	0.5	1.5	34.94[2]
Casein	15.5	4.1	22.5	4.1	8.0	10.8	8.5	3.1	5.1	6.8	4.5	1.0	5.4	7.8	76.53
Miscellaneous feed supplements															
Animal feed yeast	8.3	2.0	5.4	1.4	3.4	3.4	3.3	1.5	2.6	4.2	2.4	0.6	1.0	2.9	55.38
Irradiated dried yeast	8.8	2.2	6.3	1.2	3.8	4.1	4.8	0.7	2.3	3.0	2.8	0.6	1.0	3.0	55.16
Dried buttermilk	6.2	1.2	7.0	1.0	2.9	3.7	2.6	0.8	1.6	1.5	1.7	0.5	1.1	3.0	57.56
Dried skimmilk	6.4	1.2	7.3	1.3	2.9	3.7	2.9	0.9	2.0	1.7	1.7	0.4	0.9	2.4	58.77
Dried whey	2.7	0.3	1.7	0.2	1.0	1.2	0.8	0.2	0.5	0.6	0.8	0.1	0.1	0.8	37.82
Dried cheese whey	2.5	0.2	1.9	0.2	1.0	1.2	0.9	0.1	0.3	0.4	1.9	0.1	0.1	0.7	45.01
Dehydrated alfalfa	3.1	0.6	1.4	0.4	1.0	1.2	0.7	0.2	0.9	0.6	0.7	0.2	0.3	0.8	37.85
Dehydrated grass	3.1	1.3	3.8	0.6	1.8	2.6	1.4	0.4	1.7	1.2	1.3	0.4	0.6	2.0	81.20
Grass juice extract	5.3	0.3	1.5	0.2	0.6	0.7	0.7	0.1	0.3	1.1	0.6	0.1	0.2	0.8	14.91
Water-soluble fraction of grass	2.7	0.1	3.2	0.2	0.2	0.3	0.3	0.1	0.1	0.6	0.3	0.1	0.2	0.3	24.21

---

[1] Glutamic acid values were determined with *S. faecalis* R using $l(-)$ glutamic acid as the standard. For discussion of these values see Baumgarten, Mather, and Stone (1945).

[2] Nitrogen accounted for by 12 amino acids, as glutamic acid was not determined in this material.

The amino acid composition of tankage, meat scraps, and whole corn (Block and Bolling, 1943) and of yeast and casein (Block and Bolling, 1945a) has been investigated. In the earlier publication leucine and isoleucine were determined chemically, and in the second report the results were obtained by microbiological assay. Our results agreed well with the microbiological but not with the chemical values. The results on the other amino acids compared favorably.

Several procedures have been employed in the determination of tryptophane. Dried yeast was found to contain by chemical analysis 1.1% tryptophane (Kratzer, 1944; and Ryan and Brand, 1944) and 1.32% (Greene and Black, 1944) by microbiological assay, while our value was 1.15%. All results were calculated to 16% nitrogen for comparison. Our microbiological values agreed well with the other chemical tryptophane values of Kratzer.

Our microbiological assay values compared well with results obtained by Ryan and Brand (1944) for tyrosine and leucine; by Kuiken and co-workers (1943) for leucine, isoleucine, and valine; by Grau and Almquist (1943) for methionine.

Serine, although nonessential, has been included in this study because heretofore very few values for it have been reported.

Results obtained by microbiological assay compared favorably with recent chemical values. It has been stated (Stokes and Gunness, 1945; and Dunn *et al.*, 1944) that microbiological assays should be applied only to pure proteins and not to complex substances such as feed materials investigated by us, as some inhibiting or stimulatory substances possibly affecting the assay might be present. However, the possible effect of such interfering substances was eliminated to a large extent by assaying at different levels of concentration of the hydrolyzates. Different values should result at the various levels in an unsatisfactory assay, but this was not the experimental result. Recovery results are an additional safeguard to rule out such a possibility. It may be argued that biological methods are more specific than chemical methods, as biological systems in general respond to a specific nutrilite, whereas in colorimetric analysis of amino acids related compounds such as amines can produce a similar color that is impossible to differentiate. For instance, in tryptophane determination the color-producing compound is indole.

Undoubtedly both the chemical and biological methods are of value, but for comparative values on practical proteins the microbiological technique appears to be of greater worth. A large number of samples can be analyzed at one time and such comparative values are of great reliability.

Nutritional Significance of Results. The qualitative amino acid requirements for rats, chicks, and human beings have been established. Rose (1938) has shown 10 amino acids—arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine—indispensable for the rat. The chick requires in addition glycine (Hegsted, 1944; Almquist and Grau, 1944); the status of the glutamic acid is uncertain. Human beings require isoleucine, leucine, phenylalanine, lysine, threonine, and tryptophane (Rose, Haines, Johnson, and Warner, 1943), while arginine and histidine are dispensable (Rose, 1945).

Almquist (1945) reported quantitative requirements for the chick: 0.9% arginine, 0.8-1.0% glycine, 0.8–1.0% isoleucine, 0.15% histidine, 1.0–2.0% leucine, 0.9% lysine, 0.9% methionine, 1.0% phenylalanine, 0.5–1.0% threonine, 0.25% tryptophane, and 1.0% valine. It is well known that cereal grains are deficient in lysine and tryptophane. Distillers' by-products are considerably higher in these amino acids and constitute a good protein source. Protein concentrates as well as animal by-products are well balanced in this respect. The milk products contain fairly high proportions of lysine and tryptophane.

From such data it should be possible to evaluate a diet or ration in the light of the amino acid requirements. In addition, however, the digestibility of the proteins ingested should be known.

Summary

An improved medium for amino acid assays with *S. faecalis* R has been described. It allows evaluation of the results by the titration of the acid produced, as under our assay conditions maximal production of lactic acid and optimal utilization of the glucose was achieved. This assay technique is a simplification of our earlier procedure

The specificity of response of the three microorganisms, *L. arabinosus*, *L. casei*, and *S. faecalis* R, to their essential amino acid and their possible replacement by metabolites or predecessors of these amino acids have been investigated.

A number of feed materials has been analyzed for 12 amino acids—arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophane, tyrosine, and valine. The results are discussed in view of the literature values and their nutritive significance is indicated.

Literature Cited

Almquist, H. J.
1945 Effective use of proteins in the nutrition of the chick. Trans. Am. Assoc. Cereal Chem. 3: 158–168.
—— and Grau, C. R.
1944 The amino acid requirements of the chick. J. Nutrition 28: 325–331.

Baumgarten, W., Garey, J. C., Olsen, M. J., Stone, L., and Boruff, C. S.
1944 A medium for obtaining maximal growth response in microbiological assays of amino acids. J. Am. Chem. Soc. 66: 1607–1609.
——, Mather, A. N., and Stone, L.
1945 Glutamic acid content of feed materials. Cereal Chem. 22: 511–521.
——, Stone, L., and Boruff, C. S.
1945 Amino acid composition of grain alcohol fermentation by-products. Cereal Chem. 22: 311–320.
Block, R. J., and Bolling, D.
1943 The amino acid yield from various animal and plant proteins after hydrolysis of the fat free tissue. Arch. Biochem. 3: 217–226.
1945 The Amino Acid Composition of Proteins and Foods. Analytical Methods and Results. Charles C. Thomas, Springfield, Illinois.
1945a A note on the amino acids yielded by yeast, sunflower seed meal, and sesame seed after hydrolysis of the fat free tissue. Arch. Biochem. 6: 277–279.
Dunn, M. S., Camien, M. N., Shankman, S., Frankl, W., and Rockland, L. B.
1944 Investigations of amino acids, peptides, and proteins. XIX. The determination of lysine in protein hydrolysates by a microbiological method. J. Biol. Chem. 156: 715–724.
Fox, S. W., Fling, M., and Bollenback, G. N.
1944 Inhibition of bacterial growth by *d*-leucine. J. Biol. Chem. 155: 465–468.
Greene, R. D., and Black, A.
1943 A microbiological method for the determination of tryptophane. Proc. Soc. Exptl. Biol. Med. 54: 322–324.
1944 The microbiological assay of tryptophane in proteins and foods. J. Biol. Chem. 155: 1–8.
Grau, C. R., and Almquist, H. J.
1945 The methionine content of feedstuff proteins. Arch. Biochem. 6: 287–294.
Hegsted, D. M.
1944 Growth in chicks fed amino acids. J. Biol. Chem. 156: 247–252.
1945 The specificity of the leucine, isoleucine, and valine requirements of *Lactobacillus arabinosus* 17-5. J. Biol. Chem. 157: 741–746.
Hutchings, B. L., Bohonos, N., and Peterson, W. H.
1941 Growth factors for bacteria. XIII. Purification and properties of an eluate factor required by certain lactic acid bacteria. J. Biol. Chem. 141: 521–528.
Kratzer, F. H.
1944 The tryptophane content of feedstuff proteins. J. Biol. Chem. 156: 507–509.
Kuiken, K. A., Norman, W. H., Lyman, C. M., Hale, F., and Blotter, L.
1943 The microbiological determination of amino acids. I. Valine, leucine, and isoleucine. J. Biol. Chem. 151: 615–626.
Rose, W. C.
1938 The nutritive significance of the amino acids. Physiol. Rev. 18: 109–136.
1945 Private communication.
——, Haines, W. J., Johnson, J. E., and Warner, D. T.
1943 Further experiments on the role of the amino acids in human nutrition. J. Biol. Chem. 148: 457–458.
Ryan, F. J., and Brand, E.
1944 A method for the determination of leucine in protein hydrolysates and in foodstuffs by the use of a *Neurospora* mutant. J. Biol. Chem. 154: 161–175.
Snell, E. E.
1945 The vitamin B_6 group. IV. Evidence for the occurrence of pyridoxamine and pyridoxal in natural products. J. Biol. Chem. 157: 491–505.
1945a The vitamin B_6 group. V. The reversible interconversion of pyridoxal and pyridoxamine by transamination reactions. J. Am. Chem. Soc. 67: 194–197.
—— and Guirard, B. M.
1943 Some interrelationships of pyridoxine, alanine and glycine in their effect on certain lactic acid bacteria. Proc. Natl. Acad. Sci. U.S. 29: 66–73.

— and Rannefeld, A. N.
1945 The vitamin B_6 group. III. The vitamin activity of pyridoxal and pyridoxamine for various organisms. J. Biol. Chem. **157**: 475–489.
Stokes, J. L., and Gunness, M.
1944 Utilization of amino acid enantiomorphus [enantiomorphs] by *Lactobacillus arabinosus* and *Lactobacillus casei*. J. Biol. Chem. **154**: 715–716.
1945 Microbiological methods for the determination of amino acids. I. Aspartic acid and serine. J. Biol. Chem. **157**: 651–659.
Teply, L. J., and Elvehjem, C. A.
1945 The titrimetric determination of "*Lactobacillus casei* factor" and "folic acid." J. Biol. Chem. **157**: 303–309.
Williams, R. J.
1941 Studies on the vitamin content of tissues. I. Biochem. Inst. Texas Bull. 4137.

EFFECT OF METHOD OF COMBINING THE INGREDIENTS UPON THE QUALITY OF THE FINISHED CAKE [1]

Gwendolyn L. Tinklin and Gladys E. Vail

Kansas Agricultural Experiment Station, Manhattan, Kansas

(Received for publication July 9, 1945)

During the last few years, new methods of mixing cake ingredients have been introduced. These vary the order of combining the ingredients and are tending to replace the conventional creaming method which has long been followed. Several methods of mixing are now recommended for commercial bakeries; for home use the trend is towards the use of short mixing times.

Several investigators have emphasized the importance of the emulsion in the batter in relation to the quality of the finished cake. Nason (1939) pointed out that when the egg was added slowly a more stable emulsion was formed. Grewe (1937) had previously made the same observation. She had also found the emulsion to be more stable when the specific gravity was greater than 0.75. Halliday and Noble (1933) stated that when the emulsion was in a stable form the cakes produced were more velvety and would retain their freshness for a longer time than those made from a batter in which the emulsion broke.

Bailey and LeClerc (1935) observed that a water-in-fat emulsion resulted when eggs and milk were added gradually during the creaming process. When these were added too rapidly, a curdled mass was formed as the emulsion changed from a water-in-fat to a fat-in-water dispersion. Collins and Sunderlin (1940) found that batters of high viscosity were associated with a water-in-oil type of emulsion and those of low viscosity with an oil-in-water emulsion. Lowe (1943) stated that thin-pouring batters produced inferior cakes. She also observed that curdled batters, if the flecks were small, often produced desirable cakes.

---

[1] Contribution No. 120, Department of Home Economics. Condensed from the thesis presented by Gwendolyn L. Tinklin in partial fulfillment of the requirements for the Degree of Master of Science, May, 1944.

Lowe and Nelson (1939) pointed out that the method of combining the ingredients affected the viscosity of the batter, and that the more viscous the batter the higher the score given the cake. They referred to work by Buel in which she found that if the batter were viscous enough to stand in waves when poured into the pan, a good cake could be expected. Pyke and Johnson (1940) made similar observations regarding the relationship between the viscosity of the batter and the quality of the finished cake. They concluded that a batter with a high specific gravity and low viscosity yielded a cake of small volume, coarse grain, and a somewhat harsh, rubbery texture which was not of the velvety type. Alexander (1931) found a direct relation between specific gravity of batter and quality of the finished cake.

A cake which has been mixed just the right amount, as described by Lowe (1943), is one in which the ingredients are well blended. It has a fine grain, thin cell walls, a smooth crumb, and a velvety feel when tasted. Undermixing, according to Nason (1939), results in a coarse, uneven texture and a cake which will stale rapidly, while overmixing gives a smaller volume and a compact texture.

The present investigation was undertaken to study the effect of different methods of combining the ingredients of a simple cake (containing fat) on various characteristics of the batters and the baked cakes. The consistency and specific gravity of cake batters prepared in various ways was determined, and related to the shortness, compressibility, and eating quality of the finished cakes.

Materials and Methods

Cake Formula. The cake formula used throughout the study was as follows:

Ingredients	Quantity (g)	Approximate measures
Fat	75	⅜ cup
Sugar	200	1 cup
Cake flour	168	1¾ cups
Baking powder	10	2½ teaspoons
Salt	1.5	¼ teaspoon
Vanilla	-	1 teaspoon
Dried whole eggs	27	4 tablespoons, packed
Water	100	6⅔ tablespoons
Milk	162.7	⅔ cup

Ingredients were as nearly identical as possible. Dried eggs from the same lot, a high-ratio vegetable shortening available to institutional and commercial bakeries, finely granulated sucrose, high-grade cake flour, grade A pasteurized whole milk obtained fresh daily, a combination sulfate-phosphate (sodium aluminum sulfate-phosphate) baking powder, distilled water, salt, and vanilla were used in all cakes.

When the dried eggs were reconstituted they were added to the water and mixed either by shaking in a bottle or by beating in a bowl. When the water was at room temperature, the eggs stood for 30 minutes before they were combined with the other ingredients. When water at 67°C was used, the eggs were readily dispersed and no hydration period was allowed.

Methods of Mixing. A total of 200 cakes was made using six general methods of mixing. Minor alterations were made in these methods to bring the total number to 20.

Cakes were made by each method five times on two different types of three-speed mixers. For purposes of comparison and discussion, the work was divided into two series. In Series I the mixing was done on a Kitchen-Aid mixer with steel bowls of five-quart capacity. This mixer had a wire whip which was used for whipping the eggs and a flat paddle used for all the other mixing. In Series II a "Model C" Hamilton-Beach mixer with a one-quart and a three-quart vitreous bowl was used; the smaller bowl was used for whipping the eggs because it was a more suitable size for the amount. The three-quart bowl was flatter and more shallow than the bowl used with the Kitchen-Aid mixer. However, with each mixer the bowls were of sufficient size to permit thorough mixing of ingredients. The amount of batter was kept constant throughout the study.

The methods of mixing were briefly as follows:

Cake-Mixer Method: Reconstituted eggs whipped on high speed, fat, sugar, and salt added. Creamed on medium speed to desired specific gravity. Sifted flour and baking powder added alternately with liquid, using low speed.

Method I. Eggs reconstituted in all of the water at 68°C.

Method II. Eggs reconstituted in ¾ of the water at 68°C, ¼ of the water added with milk.

Method III. Eggs reconstituted in all of the water at room temperature. Hot water bath at 65°-70°C used while whipping.

Method IV. Same as III except ¾ of the water used for reconstituting eggs, and the remaining ¼ added with milk.

Modified Cake-Mixer Method: Creamed fat, sugar, and salt (and water if so specified in any modification) on medium speed. Changed to low speed and finished mixing.

Method V. Eggs sifted with flour and baking powder, then added alternately with combined milk and water.

Method VI. Same as V except ¾ of the water added with milk, and ¼ added with creamed mixture.

Method VII. Same as V except ½ of the water added with milk, and ½ added with creamed mixture.

Method VIII. Same as V except ¼ of the water added with milk, and ¾ added with creamed mixture.

Method IX. Same as V except all of the water added with creamed mixture.

Method X. Eggs made into paste with ½ of the water and added to creamed fat, sugar, and salt mixture, ½ of the water added with milk.

Method XI. Eggs sifted with sugar and salt and creamed with fat, all water added with creamed mixture.

METHOD XII. Same as XI except ¼ of the water added with milk and ¾ added with creamed mixture.

METHOD XIII. Eggs reconstituted and added with milk alternately with flour mixture; ¾ of the water added with eggs and ¼ added with creamed mixture.

METHOD XIV. Eggs sifted with sugar, salt, and approximately ¼ of the flour, then creamed with fat. All water added with creamed mixture.

Four-Minute Method:

METHOD XV. Eggs reconstituted with ¾ of the water, ¼ of the water added with milk. Dry ingredients sifted together. Fat and ⅔ of the liquid added. Mixed 1 minute on low speed, then 1 minute on medium speed. Remaining liquid and reconstituted eggs added. Mixed 1 minute on low speed and 1 minute on medium.

Modified "Conventional Sponge" Method:

METHOD XVI. Eggs reconstituted in all of the water at 68°C. Fat and ½ of the sugar creamed, low speed. Reconstituted eggs and other ½ of the sugar whipped 1 minute on low speed and 4 minutes on high speed. Changed to low speed to complete mixing. Baking powder, salt, and 1 tablespoon flour sifted together, added to fat-sugar mixture. Mixed slightly. One tablespoon of liquid added. Mixed. Remaining flour and liquid added alternately. Egg-sugar mixture added.

Conventional Method:

METHOD XVII. Eggs reconstituted in all of the water. Fat creamed on high speed. Sugar added, creamed on medium speed. Reconstituted eggs added and creaming continued on medium speed to desired specific gravity. Changed to low speed; flour, salt, and baking powder sifted together and added alternately with liquid.

Modified Conventional Method:

METHOD XVIII. Same as conventional except that eggs were sifted with sugar and salt and then creamed with fat. All of the water added with creamed mixture.

Flour-Batter Method:

METHOD XIX. Eggs reconstituted in all of the water at 68°C. Fat, flour, salt, and baking powder blended on low speed. Reconstituted eggs and sugar whipped on high speed to give a meringue-like mixture. Meringue and liquid added alternately to fat-flour mixture, ½ at a time, using low speed.

Dough-Batter Method:

METHOD XX. Eggs sifted with flour, salt, and baking powder, then blended with fat. All of the water added with milk. Sugar mixed with ½ of liquid and added ⅓ at a time. Remaining liquid added ½ at a time. In Series I, with Kitchen-Aid mixer, low speed was used throughout. In Series II, with Hamilton-Beach mixer, medium speed was used while incorporating sugar and ½ of the liquid; low speed used the remainder of the time.

For all cakes in the two series, the speed of mixing was the same except as specified in Method XX.

When the first mixture was of fat and sugar with or without added egg and water, it was usually creamed to a definite specific gravity. Alexander (1931) found that this reduced creaming and mixing variables.

Tests on Batters. The specific gravity determinations were made on the batters by filling a 57 ml aluminum cup, weighing it, and computing the ratio between the weight of the batter and an equal volume of water. The consistency of the batter was determined by putting the above cup of batter into a glass funnel, on the stem of which were two marks 5.0 cm apart. The bore of the stem was approximately 8 mm in diameter. The time necessary for the batter to move from the upper mark to the lower one was recorded as the measure of consistency.

Baking. The batter remaining after the small cupful was removed was poured into an aluminum cake pan 7¾ inches square and 1⅞ inches deep and baked at 365°F (185°C) for 43 minutes in a gas oven equipped with a revolving hearth and an accurate thermostatic heat control.

Evaluation of Cakes. Approximately 20 hours after baking, the cakes were cut. Four uniform slices were cut using an apparatus similar to a miter box. The first slice was not used for testing because of the crust on one side.

The height of each cake was determined by measuring in centimeters the height of the fourth slice cut, at the outer edges, at the center, and at points one-half the distance from the center to each edge. The average of these five measurements was then recorded as the standing height of the cake.

A gram shortometer, an apparatus consisting of a modified spring balance and a remodeled laboratory balance, was used to measure in grams the force necessary to break a slice of cake one inch in thickness. This device was a modification of the one used by Bailey (1934) and was described by Kramer (1935).

After testing for shortness, one-half of each broken slice was used for testing the compressibility of the cake. The apparatus used was similar to that described by Platt and Kratz (1933).

The quality of the cake was determined by a palatability committee using a score card similar to that given by the Institute of American Meat Packers (1934).

Results

Comparison of Results with Two Mixers. The time of creaming was affected by the temperature. The specific gravity of the creamed mixture differed with the ingredients used. Variation in total time of mixing (Tables I and II) for the two mixers was largely due to difference in efficiency of the mixer in the first creaming. The Kitchen-Aid mixer with a paddle similar to that used on larger commercial models apparently incorporated a given amount of air in certain combinations of ingredients more quickly than did the household-type of mixer. In other instances the reverse was true.

Comparison of the average specific gravity of batters made by the same method with the two mixers shows that in roughly one-half of the cases the Kitchen-Aid mixer (Series I) gave the lighter batter and in the other half the Hamilton-Beach (Series II). This variation is difficult to explain.

The consistency of the batters also varied in the two series (Tables I and II), but methods which gave a thick batter with one mixer also gave a thick batter with the other.

On the whole the cakes mixed with the Kitchen-Aid mixer resembled "bakery-type" cakes. Although the volume was almost equal to that obtained in Series II the texture was more compact. The comments made by the judges indicated that they considered many of the cakes to be too firm and lacking in velvetiness. Some felt harsh and bready, others were tender but tended to crumble excessively. Shortness and compressibility tests (Tables I and II) show these cakes tended to be less tender and less compressible.

The cakes in Series II were more like the typical "homemade" product. They were less compact, had a fine even grain, and were tender, but held together with little tendency toward excessive crumbling. On the whole they were smooth, soft, and velvety to the touch and on the tongue. The cakes in this series were more compressible and more tender than those in Series I, due, no doubt, to the fact that the "homemade type" was less firm and compact.

The palatability committee indicated a preference for the "homemade type" of cake as is shown by the slightly higher scores given those cakes. The cakes in Series I, the "bakery type," were given a wider range of scores than those in Series II.

Although the cakes varied considerably more in quality than is indicated by the range in palatability scores, all were edible. The palatability scores included a number of factors such as crust, shape, color of crumb, moistness of crumb, tenderness, velvetiness, and eating quality; so in many instances a lower score on one point was offset by a higher score on another. By and large, the score for eating quality tended to parallel the total palatability score. Comparison of the two series shows that the means for eating quality were exactly the same. However, if all results are considered, it is evident that no method of mixing produced cakes of identical quality when the two mixers were used. This difference may have been due to variation in beater, shape of bowl, and/or number of revolutions per minute of the beater.

Comparison of Methods of Combining Ingredients. The primary purpose of this study was to compare methods of combining the ingredients in a simple cake containing fat. These results are summarized in Tables I and II.

Each method tended to give a characteristic batter, ranging from one so thin that it could be easily poured to one so viscous that it needed to be pushed into the corners of the baking pan. The difference in consistency was determined by the time required for a given volume of batter to flow through a distance of 5.0 cm in the stem of a funnel. This varied from 0.2 minute for the thinnest batter to 52.8 minutes for the thickest. Tables I and II give averages for the different methods.

TABLE I

SERIES I. EFFECT OF METHOD OF MIXING UPON CHARACTERISTICS OF BATTER AND BAKED CAKE

Method		Batter				Baked cake			
		Time of mixing	Specific gravity	Final appearance	Consistency	Standing height	Shortness	Compressibility	Palatability score[1]
		min			*min*	*cm*	*g*	*mm*	
Cake-mixer	I	16.0	.8421	Fairly thick	10.4	5.2	125	4.3	57.8
	II	13.0	.8035	Fairly thick	11.6	5.3	124	4.3	57.8
	III	15.0	.8316	Medium thick	5.1	5.3	129	3.9	56.8
	IV	13.0	.8228	Medium thick	4.3	5.1	125	4.0	57.9
Modified cake-mixer	V	23.0	.8000	Thick[2]	17.6	5.0	112	3.8	56.9
	VI	23.0	.7632	Thick[2]	24.3	5.0	118	3.5	57.6
	VII	22.0	.7509	Thick[2]	29.2	5.0	123	3.5	57.3
	VIII	24.0	.7526	Thick, dull[2]	25.5	5.1	114	3.8	58.0
	IX	25.0	.7526	Thick[2]	28.5	4.9	120	3.6	57.4
	X	16.0	.8263	Thin	2.1	5.3	139	3.9	58.2
	XI	16.0	.8474	Very thin, glossy	0.3	5.3	141	3.6	57.9
	XII	16.0	.8614	Thin, glossy	0.3	5.1	147	3.4	56.8
	XIII	21.0	.7597	Thick, viscous	21.7	5.2	108	4.2	59.0
	XIV	16.0	.8667	Thin	0.3	5.5	141	3.4	56.8
Four-minute	XV	4.0	.9667	Very thin	0.5	4.6	113	3.1	54.9
Modified "conventional sponge"	XVI	8.4	.8667	Thin, slightly curdled	3.4	4.9	109	3.5	55.8
Conventional	XVII	15.7	.7649	Thick, dull	12.3	5.3	104	4.9	58.9
Modified conventional	XVIII	12.7	.7825	Thin, dull	0.7	5.4	119	3.9	58.2
Flour-batter	XIX	20.0	.9105	Medium thick	6.0	5.0	127	3.3	58.0
Dough-batter	XX	12.0	.9579	Quite thin	0.4	5.1	134	3.6	58.0

[1] Possible score 70.

[2] Curdled with first additions of liquid but became quite smooth, thick, and viscous with last portions of flour.

As a rule, when the eggs were sifted with the flour the batter was very thick. When the eggs were reconstituted before being added, the batter was generally thick. Exceptions were the Cake-Mixer Methods, in which the eggs were set in a hot water bath while whipping, and the Four-Minute Method. When the eggs were added with the sugar, a thin batter was usually obtained. This difference in consistency agrees with observations of Lowe and Nelson (1939), who found that the method of combining the ingredients affected the viscosity of the batter.

It was thought that temperature might be an important factor in determining the consistency of batters mixed by the different methods. However, the correlation between temperature and consistency was not significant (Table III).

Several batters curdled when the first portion of liquid was added (Tables I and II) but the curdled appearance disappeared and the

TABLE II

SERIES II. EFFECT OF METHOD OF MIXING UPON CHARACTERISTICS OF BATTER AND BAKED CAKE

Method		Batter				Baked cake			
		Time of mixing	Specific gravity	Final appearance	Consistency	Standing height	Shortness	Compressibility	Palatability score[1]
		min			*min*	*cm*	*g*	*mm*	
Cake-mixer	I	20.0	.7930	Fairly thick	13.8	5.2	115	4.5	58.0
	II	16.0	.8211	Fairly thick	7.9	5.3	115	4.4	58.9
	III	21.0	.8439	Medium thick	1.7	5.3	127	4.3	56.9
	IV	18.0	.8578	Medium thick	2.0	5.1	135	3.6	57.1
Modified cake-mixer	V	21.0	.8351	Thick[2]	6.7	5.0	102	5.0	60.1
	VI	19.0	.7772	Thick[2]	22.0	5.1	103	5.7	59.0
	VII	20.0	.7684	Thick[2]	14.2	5.1	104	5.8	58.8
	VIII	21.0	.7579	Thick, dull[2]	28.7	5.1	97	6.6	60.3
	IX	21.0	.7579	Thick[2]	26.5	5.0	104	5.0	59.5
	X	14.0	.7965	Thin	7.2	5.3	114	4.7	59.2
	XI	16.0	.8386	Very thin, glossy	0.5	5.3	135	4.1	57.2
	XII	15.0	.8526	Thin, glossy	0.5	5.3	134	3.8	57.1
	XIII	21.0	.7667	Thick, viscous	22.3	5.1	101	4.9	58.1
	XIV	17.0	.8351	Thin	0.7	5.2	131	4.0	57.8
Four-minute	XV	4.0	1.0298	Very thin	0.2	4.6	105	3.1	55.7
Modified "conventional sponge"	XVI	10.4	.9456	Thin, slightly curdled	1.3	4.7	115	3.1	56.5
Conventional	XVII	16.7	.7684	Thick, dull	13.1	5.2	108	4.2	58.1
Modified conventional	XVIII	11.7	.7947	Thin, dull	1.3	5.3	116	4.1	58.0
Flour-batter	XIX	18.0	.9053	Medium thick	3.9	5.0	131	3.5	57.8
Dough-batter	XX	12.0	.9158	Quite thin	0.4	5.1	132	3.5	58.5

[1] Possible score 70.

[2] Curdled with first additions of liquid but became quite smooth, thick, and viscous with last portions of flour.

TABLE III

CORRELATION COEFFICIENTS FOR SELECTED DATA OBTAINED FROM BATTERS AND BAKED CAKES[1]

Correlation between	Coefficient of correlation (r)	
	Series I	Series II
Specific gravity and consistency	−.66**	−.60**
Specific gravity and palatability	+.13	−.49**
Shortness and compressibility	−.54**	−.61**
Compressibility and palatability	+.16	+.71**
Shortness and palatability	−.33**	−.42**
Consistency and palatability	−.03	+.39**
Consistency and shortness	−.30**	−.54**
Consistency and compressibility	+.01	+.52**
Index to volume and specific gravity	−.21*	−.50**
Compressibility and specific gravity	−.27**	−.67**
Consistency and temperature of batter	+.19	−.10
Eating quality and palatability	+.64**	+.81**
Eating quality and other palatability factors	+.74**	+.72**
Specific gravity and shortness	+.26**	+.33**
Index to volume and consistency	−.29**	+.00
Index to volume and palatability	+.38**	+.24*
Eating quality and compressibility	+.25*	+.54**

[1] 100 cakes in each series. Calculations according to Snedecor (1940).

* Significant values.

** Highly significant values.

batter became smooth and thick when the dry ingredients and remaining liquid were added. The Modified "Conventional Sponge" Method resulted in a curdled finished batter. Some batters are described as "thick and dull" and others as "very thin and glossy." This difference in appearance may possibly be explained by the work of Collins and Sunderlin (1940), who found that the viscosity of the batter varied with the type of emulsion when the proportion of ingredients was constant. Thin batters, these workers believed, were associated with oil-in-water emulsions and thick ones with water-in-oil emulsions. Lowe (1943) states that in addition to these two types of emulsions, fat may be distributed in the cake as films, pools or lakes, or absorbed on the starch and proteins of the crumb; it may be at the cake/air interface; or it may be distributed by a combination of all or some of these. A microscopic examination of the batters in this study showed differences in the way in which the ingredients were distributed and in the number, size, and distribution of the air cells. However, it was not possible to classify these batters on the basis of type of emulsion or method of distribution of fat.

Relationship between Physical Properties of Batter and Cake Characteristics. An endeavor was made to find a correlation between certain physical properties of the batters and characteristics of the baked cakes (Table III). A highly significant negative correlation was found between specific gravity and consistency. This might indicate that the amount of air incorporated in the batter may be an important factor in determining the consistency of the batter. In the literature, emphasis has been placed upon the distribution of the fat in the batter as the factor important in determining consistency. The amount and distribution of the air seems to have been ignored.

The only other factor tested which correlated with consistency in both Series I and II was shortness. Here a highly significant negative correlation was found.

Undoubtedly the variation in specific gravity in the batter was dependent upon the amount of air incorporated in it. This ranged from 0.7368 to 1.0351 for the individual cakes and from 0.7509 to 1.0298 for different methods. Specific gravity apparently was closely related to physical characteristics of the cake. There was a significant negative correlation between specific gravity and index to volume in Series I and a highly significant negative correlation in Series II. There was a highly significant negative correlation between specific gravity and compressibility and a highly significant positive correlation between specific gravity and shortness. Thus it would appear that a light well-aerated batter is much more likely to produce a cake with desirable physical characteristics than is a heavy one. It would also seem that even though specific gravity and consistency are highly correlated, specific gravity is a better indication of cake quality than is consistency.

The standing height of the baked cakes which was used as an "index of volume" varied from 4.6 to 5.5 cm. Roughly this represented a range from 1750 cc to 2090 cc in volume, a considerable difference. The smallest cakes were made from thin batters, but interestingly enough the largest ones were also obtained from thin batters. This is not wholly in agreement with Berrigan (1937) who found that cake batters having a low viscosity produced cakes of small volume and poor texture. As previously mentioned, there was a correlation between specific gravity of the batter and volume, but in Series I it was just significant. In Series I there was a highly significant correlation between "index of volume" and palatability and in Series II a significant one. Thus it would appear, as the commercial baker frequently asserts, volume is an important consideration to many consumers of bakery goods.

Shortness, the ease with which a slice of cake can be broken, showed a highly significant correlation with specific gravity. It showed a highly significant negative correlation with consistency, with compressibility, and with palatability. Compressibility was apparently not as dependable a measure of quality as was shortness. Only in Series II was there a highly significant correlation between compressibility and palatability. When only eating quality was considered there was a correlation with compressibility in both series, although it was highly significant only in Series II. Lowe (1943) has reported that the degree of compressibility varies with the method of mixing. Cakes mixed for a short period had a coarse, loose, porous texture, and were more compressible than cakes mixed for a longer time.

As already mentioned, the palatability committee evaluated a number of characteristics as well as eating quality. The correlation between eating quality and other factors scored by the committee and between eating quality and total palatability score was very high (Table III). Frequently, however, the comment was made that a cake which was otherwise of high quality "balled up" in the mouth. Hence, a cake which scored high in other characteristics might be marked down on eating quality because of the feel in the mouth.

Summary

Batters with a wide range in consistencies can be made from the same ingredients by varying the method of combining the ingredients.

Consistency and specific gravity were found to be closely related, but consistency seemed to be a less reliable indication of the quality of the finished cake than did specific gravity.

Cake batters of low specific gravity tended to give cakes of good volume which were tender and compressible.

Very quick methods of mixing gave inferior cakes, but those methods requiring the most time did not necessarily give the best results.

Shortness and compressibility showed a highly significant correlation, but shortness appeared to be a better index to quality of the cake than was compressibility.

Two physical characteristics, index-to-volume and shortness, were correlated with the palatability score.

Literature Cited

Alexander, G. L.
1931 Creaming for a definite time compared with creaming to a definite specific gravity. Cereal Chem. **8**: 254 255.
Bailey, C. H.
1934 An automatic shortometer. Cereal Chem. **11**: 160-163.
Bailey, L. H., and LeClerc, J. A.
1935 Cake and cake making ingredients. Cereal Chem. **12**: 175-212.
Berrigan, D. G.
1937 The use of lard in cake baking. Cereal Chem. **14**: 525-531.
Collins, O. D., and Sunderlin, G. L.
1940 Cake batter viscosity as related to batter structure. J. Home Econ. **32**: 556.
Grewe, E.
1937 The emulsion-foam produced by agitating butter, sugar, and egg: a method for testing the stability of the emulsion and the effect of the conditioning temperature of the fat. Cereal Chem. **14**: 802-818.
Halliday, E. G., and Noble, I.
1933 Hows and Whys of Cooking. Univ. Chicago Press, Chicago, Ill.
Institute of American Meat Packers.
1934 Lard. Department of Scientific Research.
Kramer, R.
1935 Creaming properties of various fats as related to the specific gravity of the batter and to the quality of plain cake. Unpublished thesis. Kansas State College of Agriculture and Applied Science. 46 p.
Lowe, B.
1943 Experimental Cookery (3rd ed.). John Wiley and Sons, Inc., New York, N. Y.
— -- and Nelson, P. M.
1939 The physical and chemical characteristics of lards and other fats in relation to their culinary value. II. Use in plain cake. Iowa State Coll. Agr. Expt. Sta. Research Bull. 255: 147-213.
Nason, E. H.
1939 Introduction to Experimental Cookery. McGraw-Hill, New York, N. Y.
Platt, W., and Kratz, P. D.
1933 Measuring and recording some characteristics of test sponge cakes. Cereal Chem. **10**: 73-90.
Pyke, W. E., and Johnson, G.
1940 Relation of mixing methods and a balanced formula to quality and economy in high-sugar-ratio cakes. Food Research **5**: 335-359.
Snedecor, G. W.
1940 Statistical Methods (3rd ed.). Iowa State Coll. Press. Ames, Iowa.

STUDY OF SOME FACTORS INFLUENCING THE OXIDATION OF THIAMINE TO THIOCHROME

H. A. WATSON

Food and Drug Laboratory,
Department of National Health and Welfare, Winnipeg, Manitoba, Canada

(Received for publication May 22, 1945)

Various methods of carrying out the ferricyanide oxidation of thiamine to thiochrome are recorded in the literature. Jansen (1936), who originally suggested this reaction as a method of estimation of thiamine, described three procedures, one for pure thiamine, another for the international adsorbate, and a third for yeast extract. The order of addition and the concentration of his reagents varies. Hennessy and Cerecedo (1939) used another concentration of alkali and a different quantity of sample, and directed that the ferricyanide be added before the alkali. Harris and Wang (1941) added the reagents in this order, but claimed only 50% recovery of thiamine unless methanol was added. The method given in *Cereal Laboratory Methods* (4th ed., 1941) published by the American Association of Cereal Chemists is essentially the method of Hennessy and Cerecedo (1939), in which the addition of the ferricyanide is made before that of the alkali.

Nicholls, Booth, Kent-Jones, Amos, and Ward (1942), Wang and Harris (1942), and Glick (1944) recommend the addition of the reagents in the reverse order, that is, the alkali before the ferricyanide, Glick recommending rapid addition from a pipette with large orifice. Pyke (1939) adds the sample to a mixture of methanol, alkali, and ferricyanide, but Booth (1940) claims incomplete oxidation of the thiamine by this premixing of reagents.

Conner and Straub (1941) and Merck and Company (1941) recommended a simultaneous addition of alkali and ferricyanide by the use of a premixed reagent. This procedure was also advised by the Research Corporation Committee (1941) and by Hoffer, Alcock, and Geddes (1943). In the two latter papers mention is made that the reagent should be added rapidly.

Andrews (1944) compared results of collaborative tests made by several of these procedures, after a canvass of 42 laboratories to determine the weaknesses in the method. It was found that the concentration of ferricyanide and the order of adding ferricyanide and alkali had some effect upon the assay, and that it was preferable, particularly where a considerable excess of ferricyanide was employed, to add the alkali first followed immediately by the ferricyanide.

The quantities of ferricyanide recommended in these methods vary rather widely. Conner and Straub (1941) found that such variations produced very little effect on fluorescence if optimal amounts of sodium hydroxide were present, while other workers, e.g., Hoffer *et al.* (1943) record considerable quenching of the fluorescence with increased amounts of ferricyanide.

The clarification of isobutanol for reading in the fluorometer is carried out in a number of ways, but most commonly by the use of anhydrous sodium sulfate. Some workers specify the quantity to be used, but generally the amount recommended is somewhat uncertain. Brown, Hamm, and Harrison (1943) reported that certain samples of sulfate imparted fluorescence to the isobutanol and warned against inaccuracies from this source.

Early experience in our laboratory with the thiochrome method had proved disappointing, as consistent results could not be obtained. Efforts to improve the replicability of the test led to a suspicion that the main difficulty was connected with the oxidation step and some of the various procedures for carrying out this step were therefore submitted to a critical study.

Experimental

All strengths of sodium hydroxide solution used are referred to as percentages by weight unless otherwise stated. Details of the oxidation procedures used will be described later. After oxidation, 15 ml of isobutanol were added, the mixture was shaken for 1 minute, and then centrifuged. The aqueous layer was rejected and the isobutanol cleared with 1 ml of ethanol. Its fluorescence was then read from the potentiometer scale of a Model 11 Coleman Spectrophotometer with mercury vapor lamp and other attachments.

Greater fluorescence was invariably obtained when the sodium hydroxide was added before the potassium ferricyanide than was the case when the order was reversed, yet sodium hydroxide itself is known to destroy thiamine. When 5 ml of neutral thiamine solution and 3 ml of 15% sodium hydroxide solution are used, the resulting mixture is about 1.5 *N* with respect to sodium hydroxide. Tests were made in which smaller quantities of 15% sodium hydroxide solution were added to 5 ml aliquots of standard solution containing 1 μg thiamine and 1 mg of ferricyanide. Normal fluorescence readings were obtained with 2 ml and 1 ml of sodium hydroxide solution, but with 2 drops of sodium hydroxide solution the readings were very low, and were not increased by the addition of 3 ml of sodium hydroxide solution a few seconds after the 2 drops had been added. No increase of fluorescence occurred either, if after adding the 2 drops of sodium hydroxide solution, the solution was then made slightly acid before adding 3 ml of the alkali.

Buffer Experiments. To study this reduction of fluorescence more closely, oxidations were made at various pH levels using Sörensen's glycine buffers. Standard thiamine solution, 1 μg in 0.5 ml, was added to 4.5 ml buffer containing 0.9 mg of ferricyanide, followed 2 minutes later by 3 ml of 15% sodium hydroxide solution. Similar exposure was also made to the buffer alone for 2 minutes, the alkali then being added, followed immediately by the ferricyanide. The results are shown in Figure 1.

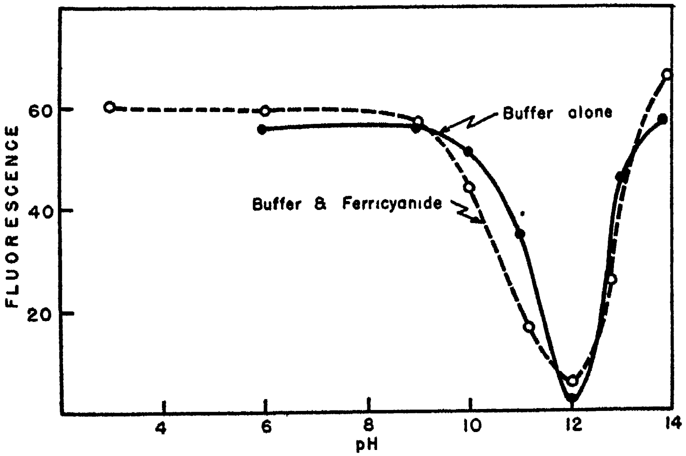


Fig 1 Effect on fluorescence of exposure of thiamine for 2 minutes to buffer solutions at different pH levels, before and after addition of ferricyanide. Fluorescence values were obtained from potentiometer drum, blank deducted, using Coleman Model 11 Spectrophotometer set at 80 with quinine sulfate (0.27 μg/ml).

The general shape of the curves was confirmed in the absence of buffer by adding increasing amounts of very dilute alkali to the thiamine solution and checking the pH.

Since only a small amount of alkali is required to carry an unbuffered solution through the pH range shown in Figure 1, the thiamine solution was next exposed for 2 minutes to varying amounts of 15% sodium hydroxide solution together with ferricyanide. At the end of the 2 minutes, sufficient alkali was added to bring the total amount up to 3 ml and the fluorescence measured (Figure 2).

The dotted part of the curve represents data obtained with buffers to give a clearer idea of the results obtained at the different levels of alkalinity using 0 to 3 ml sodium hydroxide solution. During an actual oxidation, a variable loss of fluorescence from the point marked A seems probable, depending on speed of addition of the alkali and whether the mixture is agitated or not.

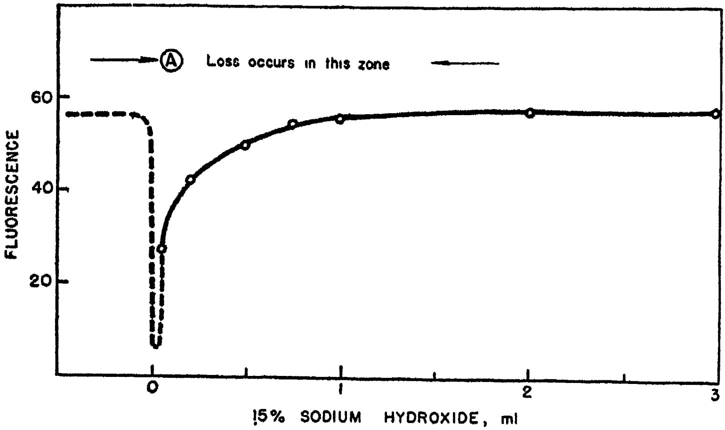


Fig. 2. Effect on fluorescence of exposure of thiamine for 2 minutes to increments of 15% sodium hydroxide solution, in presence of ferricyanide. Fluorescence values from drum of Coleman Model 11 Spectrophotometer, blank deducted, set at 80 using quinine sulfate (0.27 μg/ml).

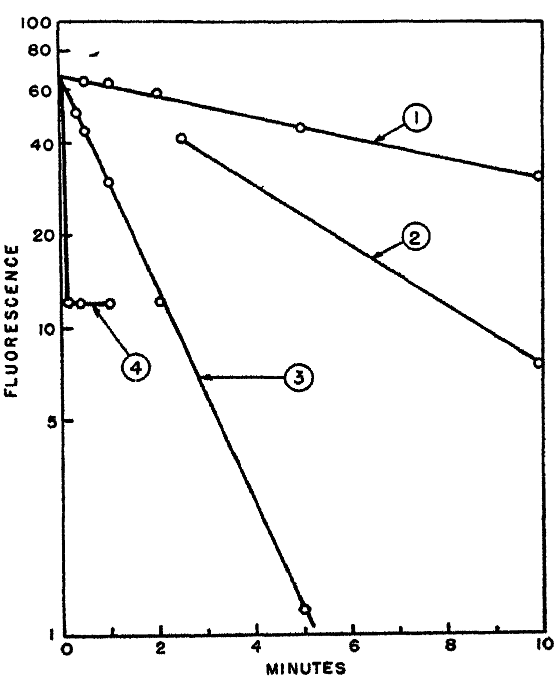


Fig. 3. Effect on fluorescence of exposure of thiamine for varying lengths of time to different strengths of alkali. Comparison with effect using buffer at pH 12 with ferricyanide. Fluorescence values from drum, Coleman Model 11 instrument, blank deducted, set at 80 with quinine (0.27 μg/ml), are plotted on a log. scale.

Curve 1.	3 ml 15% NaOH by wt.	Room temp. 25°C.[1]
Curve 2.	3 ml 30% NaOH by wt.	Room temp. 21.5°C.[1]
Curve 3.	1 drop 0.1 *N* NaOH	Room temp. 23°C.[1]
Curve 4.	Alkaline buffer (pH 12) and ferricyanide.	Room temp. 23°C.

[1] Room temperature differed somewhat for each curve, but the order of the curves and the sharp drop of curve 3 was confirmed by repeating certain points on each curve at a common temperature.

Effect on Fluorescence of Exposure of Thiamine to Alkali of Different Strengths, for Varying Lengths of Time. The similarity of the curves in Figure 1 shows that mild alkali alone has much the same effect on fluorescence as when mild alkali and ferricyanide are used. To study these effects, three series of experiments were carried out at room temperature. In two of these, 5 ml of thiamine solution (pH about 6) were exposed to 3 ml of 30% and 3 ml of 15% sodium hydroxide solution respectively for various time intervals up to 10 minutes, and ferricyanide was then added. In the third series, very weak alkali was used, 1 drop of 0.1 *N* sodium hydroxide solution acted on 5 ml of thiamine solution for the time stated, after which 3 ml of 15% sodium hydroxide solution was added, followed rapidly by the ferricyanide. The results are shown in Curves 1, 2, and 3 of Figure 3.

Action of the very weak alkali (Curve 3) is more rapid than that of the 15% or the 30%, the fluorescence falling nearly to zero in 5 minutes. Of the stronger solutions, the 30% alkali (Curve 2) acted more rapidly than the 15% alkali (Curve 1). The order of these curves indicates that in weak alkali, action may proceed along a different path from that in the strong alkali. Tests were made to see if thiamine was actually destroyed when acted on by weak alkali. After allowing thiamine solution to be acted upon by 1 drop of 0.1 *N* alkali for 5 minutes (corresponding to the lowest point on Curve 3), 2 drops of 0.1 *N* acid were then allowed to act for the same length of time, before adding 3 ml of 15% alkali and ferricyanide. Almost all of the lost fluorescence was restored. In contrast, in the earlier experiment in which ferricyanide was present during the mild alkaline treatment, no reversibility was obtained after acidifying.

Curve 4, Figure 3, is inserted to show how much more rapid is the effect produced when ferricyanide and mild alkali both act together. Thiamine added to a mixture of ferricyanide and buffer of pH 12 for only 10 seconds before addition of 3 ml of 15% alkali resulted in a striking loss of fluorescence which remained the same when repeated at 20- and 60-second intervals.

Comparison of Fluorescence Obtained by Commonly Used Methods. The three methods of oxidation commonly in use, one in which the ferricyanide is added before the alkali (Method 1), a second in which the order is reversed (Method 2), and a third in which these reagents are premixed before making the addition (Method 3), were compared using 5 ml of thiamine solution containing approximately 1 μg, 3 ml of 15% sodium hydroxide solution, and an oxidation interval of 30 seconds. The manner of making the additions was varied by pouring both slowly and rapidly with and without agitation.

A fourth method (Method 4a) based on the experiments reported in this paper was also examined. In this method the thiamine solution was added to the reagents, rather than adding the reagents to the sample. The freshly made, mixed reagent was kept well agitated during the addition from a pipette (draining in about 15 seconds) of the 5 ml of thiamine solution. From this point, the procedure was the same as for the others.

A slight modification of this procedure (Method 4b) was introduced by pouring the thiamine solution into the alkali, instead of into a mixture of alkali and ferricyanide, the oxidation then being carried out by the addition of the ferricyanide immediately after. These results are summarized in Table I.

TABLE I

COMPARISON OF FLUORESCENCE OBTAINED UPON OXIDATION OF THIAMINE BY VARIOUS METHODS

(Level of alkalinity is 3 ml of 15% sodium hydroxide)

Manner of addition	Reagent into thiamine			Thiamine into reagent	
	Method[1]			Method 4[1]	
	1	2	3	a	b
	Fluorescence[2]				
Slow, with shaking	35.0	60.0	56.0	64.0	61.5
Slow, without shaking	47.5	61.0	57.5	64.0	61.5
Fast, with shaking	54.0	61.0	60.0	62.0	60.0
Fast, without shaking	57.5	61.5	62.5	63.5	60.5
Mean	48.5	60.9	59.0	63.4	60.9

[1] Method 1. Ferricyanide added to sample before alkali. Method 2. Alkali added first, followed by ferricyanide. Method 3. Mixed reagent added to sample. Method 4. (a) Sample poured into mixed reagent. (b) Sample poured into alkali, followed by ferricyanide.

[2] Fluorescence values obtained from potentiometer drum, blank deducted, using Coleman Model 11 Spectrophotometer set at 80 with quinine sulfate (0.27 μg/ml).

Adding the ferricyanide before the alkali (Method 1) gave low and variable results. By reversing the order (Method 2), higher and more uniform results were obtained, whereas the mixed reagents (Method 3) gave intermediate values. Method 4a gave the highest fluorescence with small variability, while Method 4b showed results similar to those of Method 2.

Since the amount and strength of alkali, namely 3 ml of a 15% solution, are so commonly used, it might be supposed that this quantity produces maximum fluorescence. Tests indicated, however, that this was not the case. Using procedure 4a, 5 ml aliquots of thiamine solution were poured into 3 ml of alkali of higher strengths, but containing the same amount (1 mg) of ferricyanide. The following results were obtained.

Sodium hydroxide % by wt	Fluorescence as % (approx.)
15	100
30	114
36	118
42	118
48	118

Three ml of 36% by weight sodium hydroxide solution gave a maximum value.

Effect of Varying the Amount of Ferricyanide. A brief study was made of the effect on fluorescence of using differing amounts of ferricyanide. Several series of tests using 1 μg of thiamine in 5 ml of solution were carried out using different methods of oxidation with the results shown in Table II.

TABLE II

EFFECT ON FLUORESCENCE OF VARYING AMOUNTS OF FERRICYANIDE

Ferricyanide added	Fluorescence as percentage of maximum		
	Method 3	Method 4a[1]	Method 4b
mg			
0.1	100	100	100
0.3	94	—	–
0.5	92	-	100
1.0	87.5	100	100
2.0	82	100	100
3.0	- -	100	98
4.0	—	97.5	95
5.0	70.5		92
8.0	--	94.5	
10.0	69	-	90.5

[1] 33% alkali used; other methods 15%.

The most desirable method to use in assays where extraneous oxidizable material may be present is one permitting considerable latitude in the amount of ferricyanide added without affecting fluorescence. On this basis Method 4a using 33% alkali, or Method 4b using 15% alkali, gives satisfactory results.

Fluorescence Attributable to Anhydrous Sodium Sulfate. Early in the course of these studies erratic results were occasionally obtained, and this difficulty was traced to the presence of fluorescent material in the sodium sulfate. A study of this aspect of the method was therefore made.

Four lots of one brand, together with two other brands, all well known and of analytical grade, were examined. Tests were made by shaking with the isobutanol separated from alkali and water as in the usual blank test. Fluorescence was measured after centrifuging to remove any tiny crystals stated in Merck and Company's method (1941) to enhance fluorescence. Two strengths of quinine were used to obtain instrument settings corresponding to those employed when measuring 0.5 µg and 1.0 µg of thiamine. One lot of brand number 1 giving the highest reading was tested again after extraction with isobutanol and ether. The results obtained are shown in Table III, blank readings on the isobutanol being deducted in each case.

TABLE III

VARIATION OF FLUORESCENCE OBTAINED FROM DIFFERENT SUPPLIES OF ANHYDROUS SODIUM SULFATE

Brand No	Lot No	Fluorescence [3] 0.5 µg setting	Fluorescence [3] 1.0 µg setting
1	(a)	17.5	9.7
	(a) [1]	5.5	3.0
	(b)	6.5	3.5
	(c)	6.0	3.2
2		13.5	7.3
3		8.0	4.3
1	(d) [2]	4.0–8.6	2.4–5.2

[1] Extracted with isobutanol and ether

[2] This lot number examined at 2 and 1 g levels. All others at 3 g.

[3] Fluorescence units from potentiometer drum of Coleman Number 11 Spectrophotometer, blank deducted, set at 80 with quinine of two strengths (0.13 µg/ml and 0.27 µg/ml).

Wide variations were obtained in the blank values with the different lots of sodium sulfate. Part of this variation may be due to differences in the final pH of the isobutanol if varying amounts of water and alkali are extracted by different brands of sulfate, since Wokes, Organ, Still, and Jacoby (1944) showed that, with thiochrome at any rate, fluorescence varies with the pH of the isobutanol. Most of the variation must be due to fluorescent material present as impurity, since the extracted material is much lower than the unextracted.

Discussion

The comparison of the methods commonly in use for carrying out the oxidation of thiamine to thiochrome shows that when ferricyanide is added first to the sample, and then followed by alkali, low and uncertain results are obtained. If the alkali is added very slowly, a much lower result is obtained than if it is added rapidly. Even if alkali is added first, some lowering of fluorescence is produced if it is added slowly. Apparently a side-reaction takes place, the extent of which is influenced by the rapidity with which the reagents are added. Where alkali alone acts, this side-reaction is reversible, and it seems possible that it is related in some way to the reversible change noted by Williams and Ruehle (1935) when titrating thiamine electrometrically with sodium hydroxide. At a certain point where peculiar behavior was observed, it was believed that some molecular rearrangement took place. When ferricyanide is added first, the side-reaction is very rapid, and it is no longer reversible.

The studies on the action of alkali during the course of the oxidation, involving the use of buffers and allowing the alkali to act for varying periods of time, show that a zone of low alkalinity coincides with a zone of low fluorescence and that this is more marked when ferricyanide acts at the same time as the alkali.

Because dilute alkali alone has no permanent effect on thiamine, as indicated by the fact that treatment with acid restores fluorescence, it has been suggested that the action of the ferricyanide may proceed along two paths, one in mild alkali, producing nonfluorescent, irreversible compounds, the other in strong alkali favoring optimum production of thiochrome. This would explain adequately those cases where ferricyanide is present during the action. Since the rapid loss of fluorescence by action of weak alkali reported in Figure 3, Curve 3, was obtained with an oxidation procedure at high alkalinity, it would seem that whatever changes had been produced in the thiamine by the weak alkali must still have persisted after being made strongly alkaline. A slow reversion of the thiamine to its former condition may take place. In discussing these observations with Williams,[1] the author's attention was called to a paper by Zima and Williams (1940) showing formulas for the free base (2), a carbinol form (3), and the sodium salt (4). Assuming that the thiochrome is formed by oxidation of the free base rather than the carbinol, the data given suggest that weak alkali favors rearrangement to carbinol, while strong alkali represses it. Suppression of thiochrome formation by still stronger alkali might be due to predominance of the sodium salt. Whatever the explanation, it is necessary during oxidation to avoid conditions favoring low alkalinity. When alkali is added to the sample or to the sample containing ferricyanide, as is done at present by many workers, the first few drops produce such conditions. Rapid addition will, of course, reduce them to a minimum, but standardization between laboratories may present difficulties.

The obvious way to overcome the difficulty is to pour the sample slowly with agitation into the alkali or mixture of alkali and ferricyanide. By this means, the alkalinity is maintained at a high level throughout the addition, and the side-reaction is either prevented or held to a minimum. The use of still stronger alkali than 15% was found to be even more efficient in preventing this reaction.

---

[1] Williams, R. R., Bell Telephone Laboratories, Murray Hill, New Jersey, private communication.

To secure maximum fluorescence, the concentration of the alkali at the beginning and during the addition of sample is more important than the actual quantity of alkali. A study of the data of Conner and Straub (1941), and of our own results where maximum fluorescence was obtained, seems to indicate that to secure maximum fluorescence, an initial concentration of about 33% (45% by volume) of alkali and a final one of not less than about 11 or 12% are necessary. These conditions are met for a 5-ml assay sample by using 2 or preferably 3 ml of 33% sodium hydroxide solution. In their sodium hydroxide study, Conner and Straub (1941) added 1 ml thiamine solution to 1 ml of 45% by volume sodium hydroxide containing the ferricyanide, and only after oxidation was this diluted to 5 ml. These conditions were satisfactory for production of maximum fluorescence and meet the minimal values given above. In an actual assay, however, their order of addition is reversed, the concentrations are changed, and the conditions are no longer such as would necessarily give maximum values.

Experiments in which the quantity of ferricyanide was varied showed that the proposed method of pouring the sample into the reagent gave satisfactory results, a variation in amount from 0.1 mg to about 3 mg resulting in almost no lowering from the maximum fluorescence. Even with 8 or 10 mg of ferricyanide, the fluorescence decreased only a few percent. The results obtained when 33% alkali was used were better than those with 15%, the total drop in fluorescence, even with 8 mg being only about 5%.

While the use of alkali of the higher strengths has given satisfactory results on pure thiamine solutions and eluates, work on extracts obtained by shortened procedures such as that of Hoffer *et al.* (1943) may not give such favorable results. Experiments with various assay materials are necessary to ascertain the general utility of such a modification in procedure.

The variable blanks obtained with different lots of anhydrous sodium sulfate confirm the findings of Brown *et al.* (1943) that this material is not necessarily free from fluorescence. The author has discontinued its use, and prefers to add 1 ml of ethanol to the isobutanol for clearing purposes. Recovery of purified isobutanol for further use is easily made by distillation after giving two or three acid washings to remove ethanol.

Summary

When thiamine is oxidized to thiochrome by the commonly used methods, a loss of fluorescence occurs. The loss is minimized by adding the eluate slowly with agitation to a mixture of ferricyanide and 33% (45% by volume) sodium hydroxide solution. With this procedure the quantity of ferricyanide can be varied over a wide range without affecting the results.

Certain lots of anhydrous sodium sulfate, commonly used for clearing isobutanol extracts, contain fluorescent material and the use of ethanol is recommended in its place.

Acknowledgment

The author gratefully acknowledges the assistance of J. F. Blanchard and Miss W. M. Bridges in preparing the literature review. He is also indebted to Dr. F. D. White, Department of Biochemistry, Manitoba Medical College, and to the Milton Hersey Company for supplying different brands of sodium sulfate.

Literature Cited

Andrews, J. S.
1944 Report of the 1943-44 methods of analysis subcommittee on thiamine assay. Cereal Chem. **21**: 388 397.

Booth, R. G.
1940 The thiochrome method for the estimation of aneurin, with a survey of the aneurin content of wheats. J. Soc. Chem. Ind. **59**: 181-184T.

Brown, E. B., Hamm, J. C., and Harrison, H. E.
1943 Comparison of thiamine values by chemical and bioassay methods. J. Biol. Chem. **151**: 153-161.

Conner, R. T., and Straub, G. J.
1941 Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem. (Anal. Ed.) **13**: 380-384.

Glick, D.
1944 Simplified procedures for the determination of thiamine in wheat flours and bread by the thiochrome method. Cereal Chem. **21**: 119-126.

Harris, L. J., and Wang, Y. L.
1941 Vitamin methods. I. An improved procedure for estimating vitamin B_1 in foodstuffs and biological materials by the thiochrome test including comparisons with biological assays. Biochem. J. **35**: 1050 1067.

Hennessy, D. J., and Cerecedo, L. R.
1939 The determination of free and phosphorylated thiamin by a modified thiochrome assay. J. Am. Chem. Soc. **61**: 179-183.

Hoffer, A., Alcock, A. W., and Geddes, W. F.
1943 A rapid method for the determination of thiamine in wheat and flour. Cereal Chem. **20**: 717-729.

Jansen, B. C. P.
1936 Chemical determination of aneurin (vitamin B_1) by the thiochrome reaction. Rec. Trav. Chim. **55**: 1046-1052.

Merck & Co.
1941 Determination of thiamin hydrochloride by the thiochrome method with the adaptation of the Hennessy and Cerecedo procedure as carried out in the laboratories of Merck and Company, Inc. Revised June 6, 1941, Rahway, N. J.

Nicholls, J. R., Booth, R. G., Kent-Jones, D. W., Amos, A. J., and Ward, H. H.
1942 Ministry of Food. Vitamin B_1 (aneurine) assay in white flour. Analyst **67**: 15-17.

Pyke, M.
1939 The distribution of vitamin-B_1 in foods as determined by chemical analysis. J. Soc. Chem. Ind. **58**: 338-340.

Research Corporation Committee
1941 Sub-committee on fluorometric methods, D. J. Hennessy, Chairman, reported in Coleman Electric Co., New York City—Maywood, Ill. Technical Bull. F-108.

Wang, Y. L., and Harris, L. J.
1942 Further notes on the estimation of Vitamin-B_1 by the thiochrome method. Chemistry & Industry 61: 27–28.

Williams, R. R., and Ruehle, A. E.
1935 Studies of crystalline Vitamin B_1. XI. Presence of quaternary nitrogen. J. Am. Chem. Soc. 57: 1856–1860.

Wokes, F., Organ, J. G., Still, B. M., and Jacoby, F. C.
1944 The calibration of fluorimeters used for estimating vitamins, alkaloids, and other substances. Analyst 69: 1–5.

Zima, O., and Williams, R. R.
1940 Über ein antineuritisch wirksames Oxydationsprodukt des Aneurins. Ber. 73B: 941–949.

THE PHOSPHORYLASE OF WAXY MAIZE[1]

LAURA BLISS[2] and NELLIE M. NAYLOR

Department of Chemistry, Iowa State College, Ames, Iowa

(Received for publication August 21, 1944)

The enzyme phosphorylase which catalyzes the reversible reaction,

starch + inorganic phosphate $\rightleftarrows$ glucose-1-phosphate,

is a part of a complex system of enzymes which has to do with carbohydrate metabolism through phosphorylation. The mechanism formulated by Meyerhof (1935, 1941) is in part as follows:

$$\begin{array}{lcl}
\text{glycogen, starch} & & \text{d-glucose} \\
+H_3PO_4 \downarrow\!\uparrow \text{(phosphorylase)} & & +H_3PO_4 \downarrow\!\uparrow \text{(hexokinase)} \\
\text{glucose-1-phosphate} & \rightleftarrows & \text{glucose-6-phosphate} \\
\quad\text{(Cori ester)} & & \quad\text{(Robison ester)} \\
 & & \qquad\qquad \downarrow\!\uparrow \text{(isomerase)} \\
 & & \text{fructose-6-phosphate} \\
 & & \quad\text{(Neuberg ester)} \\
 & & +H_3PO_4 \downarrow\!\uparrow \\
 & & \text{fructose-1, 6-diphosphate} \\
 & & \quad\text{(Harden-Young ester)}
\end{array}$$

A study of this mechanism reveals the difficulties involved in attempting to isolate for study the one reaction of starch to glucose-1-phosphate.

The relationship among the principal reactions that may take place when glucose-1-phosphate is present in the enzyme system or when it is formed through the breakdown of starch by phosphorylase (Hanes, 1940a) is shown as follows:

---

[1] Journal Paper No. J1228 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 790; supported in part by a grant from the Science Research Institute funds of Iowa State College.

[2] Present address: Randolph-Macon Woman's College, Lynchburg, Virginia.

(1) Glucose-1-phosphate is transformed progressively, by the enzymes indicated, to reducing hexose-6-phosphates (shown by Sutherland, Colowick, and Cori, 1941, to be reversible) with a resultant decrease in inorganic phosphate.

(2) Glucose-1-phosphate is transformed by phosphorylase into a polysaccharide and inorganic phosphate is liberated; the reaction may be catalyzed by the presence of a trace of starch.

(3) The polysaccharide formed (as in 2) is hydrolyzed by amylase (if present in the system) and thus the equilibrium may be shifted to the left.

(4) Hexose-6-phosphate (as in 1) is transformed into hexose-diphosphate by the appropriate enzymes with a consequent decrease in inorganic phosphate.

(5) When the concentration of glucose-1-phosphate falls to a certain level, reaction 2 ceases; further reduction of glucose-1-phosphate (by continuance of reaction 1) causes the reversal of reaction 2 and thus brings about the reconversion of the polysaccharide into glucose-phosphate.

It is possible to vary the conditions of the reactions and the preliminary treatments of the extract and thus to alter the relative velocities of these different reactions. Hanes (1940b) showed that at pH 8.0 to 8.5 reaction 1 proceeded very rapidly at the expense of reaction 2, which became negligibly small. The opposite effect—speeding up of reaction 2 and slowing down of reaction 1—was noted at pH 6.4.

Hanes (1940a), using potato extracts, studied the effect of pH and of the concentrations of the three reactants (starch, glucose-1-phosphate, and inorganic phosphate) upon the reversibility of the reactions:

$$\text{starch} + \text{inorganic phosphate} \rightleftarrows \text{glucose-1-phosphate}$$

He concluded (1) that the final equilibrium state is dependent upon the pH of the digest; and (2) that the final equilibrium state is independent of the concentrations of the reactants or the enzymes.

Hanes (1940) made purified phosphorylase preparations from pea seeds and showed that the enzyme acts on a variety of substrates; for example, saccharides composed of glucopyranose units linked in positions 1 and 4 (as in maltose), regardless of chain length and whether in colloidal aggregation or in free form terminated by reducing groups. Hanes concluded that the action of phosphorylase on starch and dextrins consisted of an endwise attack at the nonaldehydic end of the chain structure, with the liberation of individual glucose units in the form of glucose-1-phosphate. Hanes also reported a method for the preparation of glucose-1-phosphate in good yield from starch, through the action of a partially purified phosphorylase from potatoes.

Cori, Cori, and Schmidt (1939) studied the polysaccharides synthesized through phosphorylase by the reaction:

$$\text{glucose-1-phosphate} \rightleftarrows \text{starch} + \text{inorganic phosphate}$$

They reported that the polysaccharide formed by phosphorylase from brain, heart, liver, and yeast gave a brown color with iodine and resembled glycogen, whereas the enzyme from muscle extract synthesized a polysaccharide that gave a blue color with iodine and resembled starch. Hanes (1940a) also reported a blue-staining polysaccharide synthesized by potato phosphorylase.

The studies on phosphorylase by Hanes (1940a), Cori, Colowick, and Cori (1938, 1938a, 1939), Cori, Schmidt, and Cori (1939), Cori and Cori (1943), and Green and Stumpf (1942) showed the presence of phosphorylase in a wide variety of animal and vegetable tissues, and indicated that a further study of the problem might reveal a correlation between the nature of the enzyme action in a plant tissue and the type of starch synthesized (amylose or amylopectin). Experiments indicate that waxy starches are amylopectin (Bates, 1943), and thus it is of interest to study the enzyme system of waxy maize. The experiments were planned (1) to obtain some estimate of the role of phosphorylase in the development of the waxy maize kernel, and (2) to prepare a phosphorylase enzyme concentrate from waxy maize, and (3) to study the polysaccharide formed by the action of waxy maize phosphorylase with glucose-1-phosphate.

Experimental Methods and Results

Phosphorus Determinations. By making use of the equilibrium equation, starch + inorganic phosphate $\rightleftarrows$ glucose-1-phosphate, the activity of the enzyme acting upon starch or glucose-1-phosphate was determined by measuring the decrease in inorganic phosphorus as shown by the forward direction of the reaction in which starch is the substrate, or the increase in phosphorus as indicated by the reverse reaction, in which glucose-1-phosphate is the substrate. Cori, Colowick, and Cori (1938) showed that the determination of inorganic phosphorus is justified as a procedure for measuring the formation or breakdown of glucose-1-phosphate, although the method reported by Hanes (1940) was followed in many experiments in which the free, or inorganic, phosphorus was compared with the hydrolyzed or 7-minute phosphorus determinations, representing total free + ester-phosphorus.

In all tests, the following procedure was used: An aliquot was removed from the enzymic digest and added to 5 ml of 10% trichloracetic acid (to precipitate the protein). Centrifugation cleared the mixture and an aliquot of the centrifugate was used. To this sample were added 5 ml of acidic ammonium molybdate, 2 ml of 1-amino-2-naphthol-4-sulfonic acid, and water to 50 ml volume. A blue color developed which was read exactly 20 minutes after adding the reducing agent. The method was adapted from those described by Fiske and Subbarow (1925) and by King (1932). The reading was made by means of a photoelectric colorimeter, and translated to milligrams of phosphorus by means of a calibration curve. (Corning Glass filters, No. 246 Red, 4 mm were used. These transmit light in the region of 600 to 650 mμ.)

Activity of the Enzyme during the Growth of Waxy Maize. For these studies, the waxy maize was collected from the field and ground to a pulp (or flour). Distilled water equal to the weight of corn was added and the mixture held at icebox temperature (approximately 5°C) for 18 hours. (A small amount of thymol was added to retard the action of microorganisms.) The solid matter was discarded and the liquid enzyme extract cleared by centrifugation. To remove the remaining inactive material (protein and starch) the enzyme extract was heated rapidly to 50°C and kept at that temperature for 5 minutes and centrifuged. Ammonium sulfate precipitates were made by the following procedure: 20 g of ammonium sulfate per 100 ml of extract was added while the solution was still warm, and the precipitate was centrifuged off and discarded; from the centrifugate, another fractional precipitation with 16 g of ammonium sulfate per 100 ml of extract yielded active enzyme.

The enzyme precipitate was suspended in citrate buffer and used in digestion mixtures with glucose-1-phosphate (Cori, Colowick, and Cori, 1937) as a substrate. According to the method of Green and Stumpf (1942) the activity units were determined as the amount of enzyme which catalyzes the liberation of 0.1 mg of inorganic phosphate from glucose-1-phosphate in 3 minutes at 38°C and pH 6.0.

Tests for enzyme activity were made upon samples of waxy maize collected at short intervals (1 week to 10 days) during the season of development of the corn kernel. The data from these experiments are reported in Table I. Calculated on the basis of the units of phosphorylase per gram of dry corn, the activity varied from 5.09 to 0.28 units during the period from two weeks after pollination to maturity. The trend is the same if the data are reported on the basis of the units of phosphorylase per gram of waxy maize as collected from the field; on the latter basis the data show a variation of .85 to .26 units over the same period. Although enzyme concentrates of considerable activity

TABLE I

PHOSPHORYLASE ACTIVITY IN WAXY MAIZE AT DIFFERENT STAGES OF GROWTH

Days after pollination	Moisture content	Phosphorylase units per gram	
		as collected	dry weight
	%		
16	83.2	0.85	5.09
23	68.5	0 37	1.16
30	51.5	0.45	0.95
40	42.9	0.39	0.70
51	35.0	0.48	0.74
Mature corn	6.1	0.26	0.28

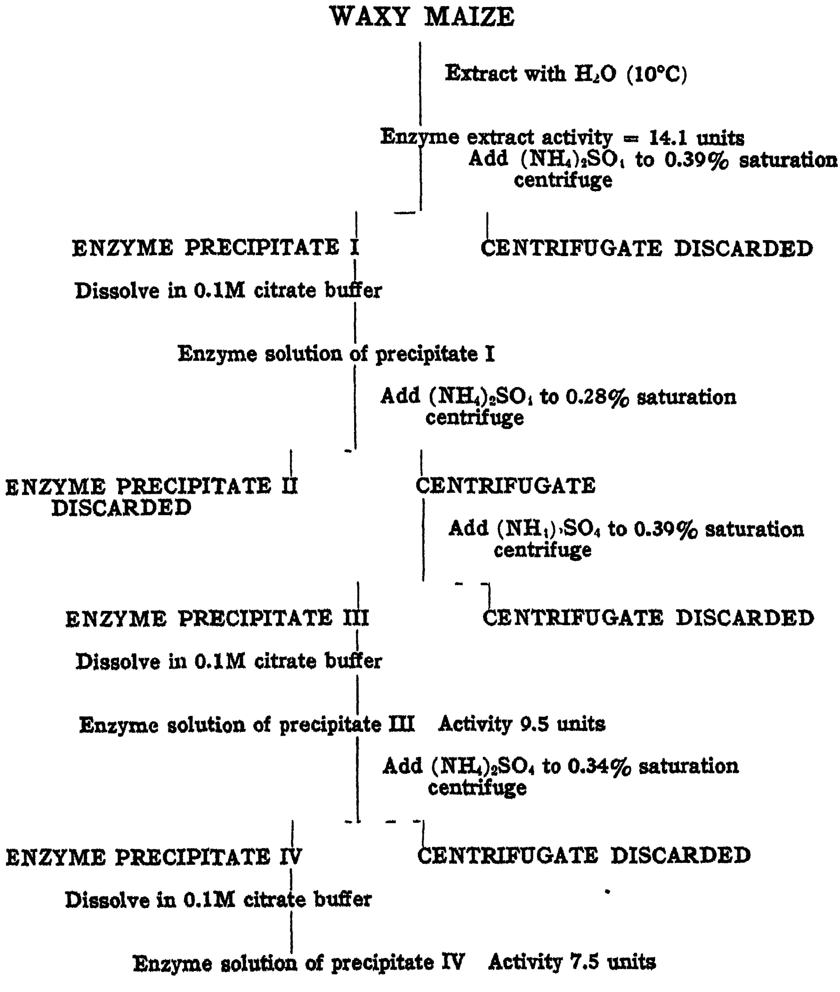

WAXY MAIZE
Extract with H_2O (10°C)
Enzyme extract activity = 14.1 units
Add $(NH_4)_2SO_4$ to 0.39% saturation centrifuge
ENZYME PRECIPITATE I
CENTRIFUGATE DISCARDED
Dissolve in 0.1M citrate buffer
Enzyme solution of precipitate I
Add $(NH_4)_2SO_4$ to 0.28% saturation centrifuge
ENZYME PRECIPITATE II DISCARDED
CENTRIFUGATE
Add $(NH_4)_2SO_4$ to 0.39% saturation centrifuge
ENZYME PRECIPITATE III
CENTRIFUGATE DISCARDED
Dissolve in 0.1M citrate buffer
Enzyme solution of precipitate III Activity 9.5 units
Add $(NH_4)_2SO_4$ to 0.34% saturation centrifuge
ENZYME PRECIPITATE IV
CENTRIFUGATE DISCARDED
Dissolve in 0.1M citrate buffer
Enzyme solution of precipitate IV Activity 7.5 units

Fig. 1. A flow sheet showing steps in making enzyme concentrates

could be obtained from corn during the later stages of growth, the data show a decrease in phosphorylase activity as the maize kernel develops.

Concentration of the Enzyme. A study was made of the methods for concentration of phosphorylase by dialysis, precipitation, and adsorption as used by Cori and co-workers (1938, 1938a, 1939), Hanes (1940), and Green and Stumpf (1942). An adaptation of these methods was used for the preparation of phosphorylase concentrates from waxy maize. Dialysis of the extract against distilled water was found to aid in forming protein precipitates which may be taken out, and to remove the inorganic phosphate originally present in the extract.

Figure 1 shows the procedure and the data obtained in making a phosphorylase enzyme concentrate. During the precipitation of the enzyme, it was necessary to work quickly and continuously, since there was deterioration of the enzyme if held for a period in the presence of ammonium sulfate. The results show that fractional precipitation with ammonium sulfate accomplishes a separation of inactive from active material, always retaining a high percentage of the original activity as measured in phosphorylase units (Green and Stumpf, 1942). Starting with an extract containing 14.1 units per 100 ml, the flow sheet indicates precipitation of enzyme at 0.39% saturation of ammonium sulfate, dissolving the precipitate in 25 ml of citrate buffer, and another fractional precipitation with ammonium sulfate in which inactive material is removed in precipitate II and the ammonium sulfate concentration increased again to 0.39% saturation to make precipitate III. When precipitate III is redissolved in 25 ml of citrate buffer, the dispersion shows 9.5 phosphorylase units, which finding indicates that 67.4% of the original enzyme activity is present in this dispersion. From this enzyme dispersion, a finer fractionation was made at 0.34% ammonium sulfate to give a final precipitate which, dissolved in 10 ml of buffer, contained 7.5 phosphorylase units. The final dispersion thus held about 53% of the original activity. Many experiments of this nature yielded corresponding results, and the dispersions were used in the experiments which follow.

A Study of the Polysaccharide Formed by the Action of Phosphorylase upon Glucose-1-Phosphate. An investigation of the polysaccharide synthesized by the action of waxy maize phosphorylase was undertaken to determine the nature of the synthetic product, and thus to compare it with the natural starch from the same source. Bates, French, and Rundle (1943) found that a potentiometric iodine titration yielded information regarding the structure of the polysaccharide. By such a titration, Bates *et al.* could estimate the proportion of each of the two starch fractions, amylose and amylopectin, present in the sample. When amylose or straight chain material was titrated, an iodine complex was formed and the iodine activity remained about the same until the complex formation was completed (giving a straight line portion to the titration curve); then the potential began to rise sharply. In contrast, amylopectin showed a steady increase in potential throughout the titration.

This technique was applied to the analysis of the small amounts of synthetic polysaccharide formed in digests containing the waxy maize phosphorylase. The digests were made up to contain the potassium salt of glucose-1-phosphate, a catalytic amount of soluble starch, citrate buffer, and the enzyme concentrate of waxy maize. (A blank determination on the catalytic amount of starch was made.) Digestion was carried out at 38°C for one-half hour. At the end of this period, the digestion was stopped by the addition of 0.5 *N* potassium hydroxide, made up to 0.05 *N* with respect to potassium iodide, diluted to 100 ml, and titrated potentiometrically. The results of two experiments (Digests A and B) are shown in Figure 2, together with the

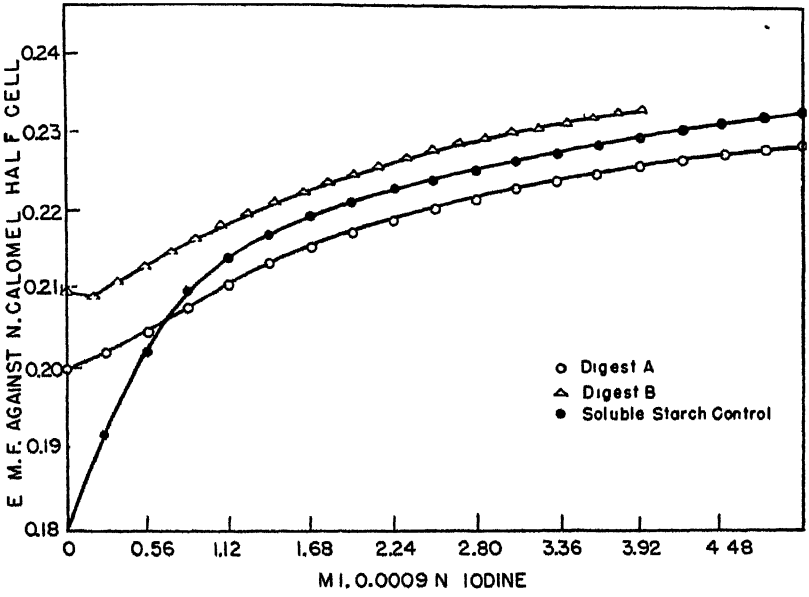


Fig 2 Iodine titration curve of soluble starch control, and of enzyme Digests A and B.

curve for the soluble starch used as a catalyst. The soluble starch contains no amylose as the curve is like that of amylopectin. The digestion curves are of a different nature and resemble those of a straight chain material which binds iodine. The high potential of the curve indicates the formation of material of short-chain length.

In another experiment (Digest D), the enzyme dispersion was prepared as previously described except that the original extract was dialyzed for 23 hours at 5°C (icebox temperature) against 0.2 *M* potassium chloride, as an additional means of purification. After dialysis, the phosphorylase was fractionally precipitated with ammonium sulfate.

This digest contained the phosphorylase which was precipitated at 0.28 to 0.39% saturation with ammonium sulfate, from 750 ml of extract; it was made up to a volume of 25 ml, to which was added 0.2 ml starch, 1 ml of 0.1 *M* potassium salt of glucose-1-phosphate, 3.8 ml of 0.1 *M* citrate buffer (making a volume of 30 ml). Digestion was carried out at room temperature. Aliquots for the determination of free-phosphorus, free-+ ester-phosphorus, and for iodine titration, were removed after 1.5 minutes, after 12 hours, and after 24 hours. The iodine titration curves are shown in Figure 3 together with that for soluble starch and a titration curve for 0.05 *N* potassium iodide solution.

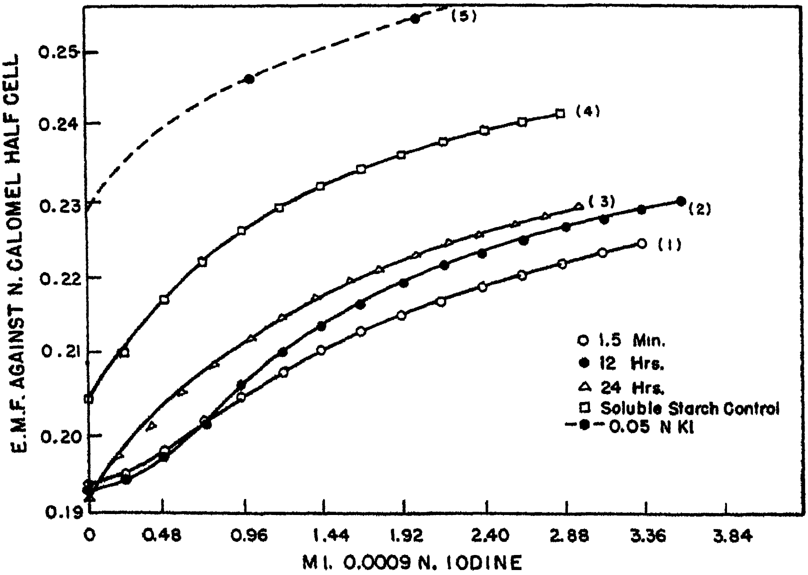


Fig. 3. Iodine titration curve of soluble starch control and of KI, together with curves for three time-interval aliquots of Digest D.

The phosphorus analyses showed an increase in inorganic phosphorus which corresponds to the synthesis of 14.3 mg of polysaccharide in the entire time of digestion. In the aliquots tested, at different time intervals, the following amounts of polysaccharide were indicated:

0	1.5 minutes	0.32 mg
0	12 hours	1.66 mg
0	24 hours	2.38 mg

The results of the iodine titration of the aliquots removed at different time intervals (Curves 1, 2, and 3, Figure 3) seem to indicate that up to 12 hours digestion at room temperature, straight chain iodine-binding material was formed, while a change in the character of the starch was apparent in the period from 12 to 24 hours, at which time the curve is of the nature of amylopectin.

These data are interpreted to indicate that the phosphorylase of waxy maize acting upon glucose-1-phosphate as a substrate liberates inorganic phosphorus with the synthesis of products of a starch-like nature, as indicated by the iodine-titration method.

Summary

Waxy maize collected from the field during growth from 16 days after pollination to maturity was tested by precipitation of the enzyme from extracts and determination of the phosphorylase units present. Data for the 1942 corn showed a considerable activity of phosphorylase in the earliest corn tested. Phosphorylase precipitates were obtained from corn at later stages of growth, and these showed decreasing activity up to maturity.

The techniques reported in the literature for the purification and concentration of the enzyme were tested to determine the method which would be most adaptable to the preparation of phosphorylase from waxy maize. Fractional precipitation with ammonium sulfate at concentrations between 0.28 and 0.34% of saturation yielded the most active precipitate.

Digests containing glucose-1-phosphate and phosphorylase of waxy maize were tested for phosphorus content, and by the iodine titration method. The data indicated the liberation of phosphorus with consequent formation of iodine-binding material of a starch-like nature.

Literature Cited

Bates, F. L.
1943 Some applications of electrometric methods to the study of the components of starch. Thesis, Iowa State College.
, French, D., and Rundle, R. E.
1943 Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. 65: 142–148.
Cori, C. F., Colowick, S. P., and Cori, G. T.
1937 The isolation and synthesis of glucose-1-phosphoric acid. J. Biol. Chem. 121: 465 477.
——, Schmidt, G., and Cori, G. T.
1939 The synthesis of a polysaccharide from glucose-1-phosphate in muscle extract. Science 89: 464 465.
Cori, G. T., Colowick, S. P., and Cori, C. F.
1938 The formation of glucose-1-phosphoric acid in extracts of mammalian tissues and of yeast. J. Biol. Chem. 123: 375–380.
1938a The action of nucleotides in the disruptive phosphorylation of glycogen. J. Biol. Chem. 123: 381–389.
1939 The activity of phosphorylating enzyme in muscle extract. J. Biol. Chem. 127: 771 782.

——, and Cori, C. F.
1943 Crystalline muscle phosphorylase. IV. Formation of glycogen. J. Biol. Chem. **151**: 57 63.
-- , --, and Schmidt, G.
1939 The role of glucose-1-phosphate in the formation of blood sugar and synthesis of glycogen in the liver. J. Biol. Chem. **129**: 629 639.
Fiske, C. H., and Subbarow, Y.
1925 The colorimetric determination of phosphorus. J. Biol. Chem. **66**: 375 400.
Green, D. E., and Stumpf, P. K.
1942 Starch phosphorylase of potato. J. Biol. Chem. **142**: 355 366.
Hanes, C. S.
1940 The breakdown and synthesis of starch by an enzyme system from pea seeds. Proc. Roy. Soc. London **B128**: 421 450.
1940a The reversible formation of starch from glucose-1-phosphate catalyzed by potato phosphorylase. Proc. Roy. Soc. London **B129**: 174 208.
1940b Enzymic synthesis of starch from glucose-1-phosphate. Nature **145**: 348-349.
King, E. J.
1932 The colorimetric determination of phosphorus. Biochem. J. **26**: 292 297.
Meyerhof, O.
1941 Biological Symposia V: 141 156 (J. Cattell Press).
— —, and Kiessling, W.
1935 Über das Auftreten und den Umsatz der α-Glycerin-phosphorsaure bei der enzymatischen Kohlenhydratspaltung. Biochem. Z. **264**: 40 71.
Sutherland, E. W., Colowick, S. P., and Cori, C. F.
1941 The enzymatic conversion of glucose-6-phosphate to glycogen. J. Biol. Chem. **140**: 309-310.

SOME EFFECTS OF CABINET FERMENTATION ON SPONGE TEMPERATURES AND DOUGH AND BREAD CHARACTERISTICS

FLOYD SCHOONOVER, JACOB FREILICH, and SUTTON REDFERN

The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.

(Received for publication July 23, 1945)

The use of cabinet fermentation has excited a great deal of interest among bakers and cereal chemists in recent years. Some have contended that great benefit is derived from the use of cabinets, within which the troughs containing the sponges are allowed to stand during sponge fermentation. Others have just as vigorously argued that the use of cabinets produces no significant changes in the sponge or in the resulting bread.

It has been our purpose to study cabinet fermentation in order to determine whether it is effective under bake shop conditions, and if so, to what extent. With this end in view, sponges were fermented in cabinets and in open troughs under varying conditions of temperature and humidity. Observations were made on sponge temperature, fermentation loss, pH of sponge, farinograph sponge and dough curves, handling of the doughs in the makeup machinery, proof time, loaf volume, and general bread characteristics, such as texture, grain, and external appearance.

Formula and Procedure

A commercial type sponge-dough formula was used, including milk powder, malt extract, and bread improver. In the sponge 30 lbs. of flour were used, and in the dough 20 lbs.; the sponge flour was a Northwestern patent and the dough flour a Kansas patent; both flours were bleached.

The sponges were mixed at the temperatures indicated, placed in open or cabinet-enclosed troughs, and fermented 2¾ to 4½ hours, usually in a controlled dough room at 80°F (26.7°C) and 96% humidity. The fermented sponge was then mixed with the balance of the dough ingredients. After a floor time of 15 minutes, the dough was put through the divider and rounder, through the molder 12 minutes later, then proofed at 96°F (35.6°C) and 90% humidity, and baked 30 minutes at 480°F (249°C) with steam in the oven.

Variations in formula or procedure were made as indicated.

Temperature measurements on some of the sponges were made with calibrated copper-constantan thermocouples, using a Leeds and Northrup portable precision potentiometer.

Farinograph curves were made with some of the sponges and doughs. The sponge curves were made at the end of sponge fermentation, just before the sponge was mixed with the balance of the dough ingredients. The dough curves were made soon after the sponge was mixed with the balance of the dough ingredients. For each curve, 520 g of sponge or dough were used in the farinograph.

Other measurements and observations were made as indicated for the individual experiments. Differences were noted as the doughs went through the makeup machines, and bread score observations were made by several qualified individuals.

Experiment No. 1. In an experiment with the dough room temperature controlled at 80°F (26.7°C) and the sponges mixed at 77°F (25°C), open trough fermentation was compared to cabinet fermentation in 4-hour sponges. At the same time, open and cabinet sponges were fermented for 2 hours and 40 minutes, under similar conditions, to check on the claim that sponge fermentation time in the cabinet may be reduced by one-third.

The results are shown in Table I.

There were no significant differences between open and cabinet fermentation, but the differences between 4 hour and 2 hour and 40 minutes sponge times were definitely significant, under the conditions outlined in Table I (temperature, fermentation loss, and proof

TABLE I

Effects of Variations in Sponge Time with Cabinet and Open Trough Fermentation

(2% yeast, 60% sponges; dough room, 80°F; sponge, 77°F)

	Final sponge temperature[1]		Sponge ferm. loss	Sponge pH	Sponge consist. after 5½ minutes (farinograph units)	Proof time	Loaf volume
	Center	Edge					
	°F	*°F*	*%*			*min.*	*cc*
4 hr. sponge; open	89.5	85.0	2.06	4.89	450	69	2,300
4 hr. sponge; cabinet	87.5	85.5	2.06	4.85	430	71	2,290
2 hr. 40 min. sponge; open	85.0	—	1.16	5.00	520	73	2,270
2 hr. 40 min. sponge; cabinet	85.0	--	1.16	5.00	520	74	2,285

[1] Sponge temperature measurements were made by means of long thermometers, both in the center, and at the edge of the sponge in contact with the trough.

time). The cabinet doughs did appear to be slightly more pliable in going through the machines, but the bread showed no difference between open and cabinet fermentation in grain, crumb color, or break and shred. The 4-hour loaves, however, were better developed and showed finer grain and more age than those from the 2 hour and 40 minutes sponge fermentation.

Figure 1 shows the farinograph sponge and dough curves for Experiment No. 1. Significant differences were obtained between the 4 hour and the 2 hour and 40 minutes sponges, as indicated by the rate of breakdown, or loss in consistency during mixing in the farinograph. But there were no differences between the open and cabinet

TABLE II

Effects of Variations in Sponge Time with Cabinet and Open Trough Fermentation

(1.5% yeast, 66% sponges; dough room, 80°F; sponge, 77°F)

	Final sponge temperature		Sponge ferm. loss	Sponge pH	Sponge consist. after 5½ minutes[1] (farinograph units)	Proof time	Loaf volume
	Center	Edge					
	°F	*°F*	*%*			*min.*	*cc*
4½ hr. sponge; open	89.75	82.25	2.07	4.90	440	72	2,350
4½ hr. sponge; cabinet	88.75	85.0	2.07	4.92	440	74	2,340
3 hr. sponge; open	84.75	—	1.0	5.14	560	85	2,360
3 hr. sponge; cabinet	84.5	—	1.0	5.16	540	88	2,350

[1] For some of the curves, the farinograph was run longer, but the values given were read at 5½ minutes in order to conform with the values for the 5½ minute curves in Figure 1.

sponges, showing that under our conditions, fermentation in the cabinet produced no measurable changes in water requirements and sponge consistency

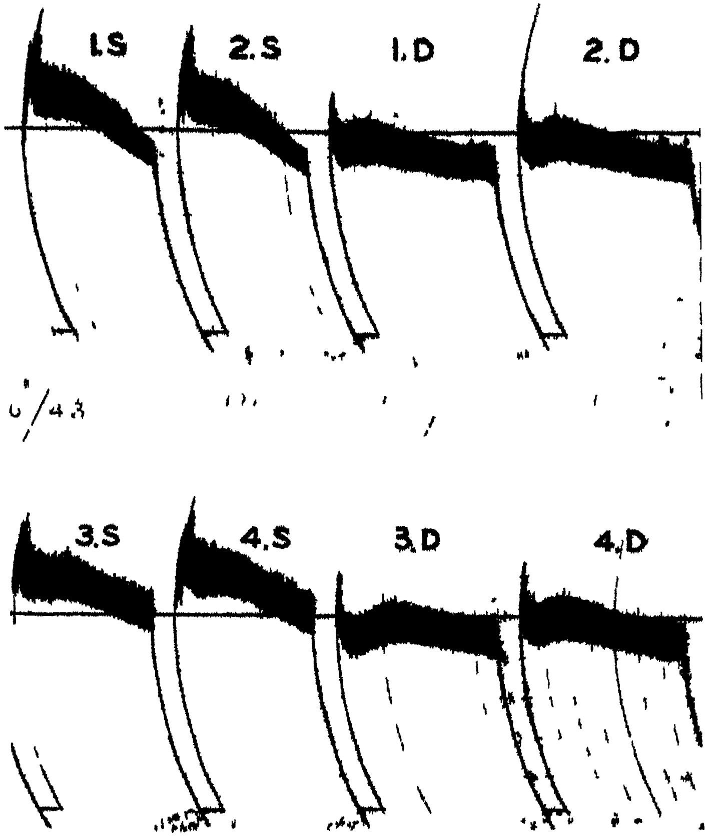


Fig 1 Effects of variations in sponge time on farinograph curves made from sponges and doughs, after sponge fermentation in cabinets and in open troughs (see Table I)

S = sponge, D = dough
1 = 4-hour sponge, open
2 = 4-hour sponge, cabinet
3 = 2-hour, 40-min sponge, open
4 = 2-hour, 40-min sponge, cabinet

The differences between the corresponding dough curves were relatively slight, but again indicated that time of fermentation rather than the use of the cabinet was the important factor here. (The experimental error in these measurements is within 20 farinograph units.)

Experiment No. 2. In this experiment, 66% sponges with 1.5% yeast were used in open trough and cabinet fermentation. The results are shown in Table II and by the farinograph curves in Figure 2. Here, again, the significant differences were due to time of sponge fermentation, and not to the use of the cabinets.

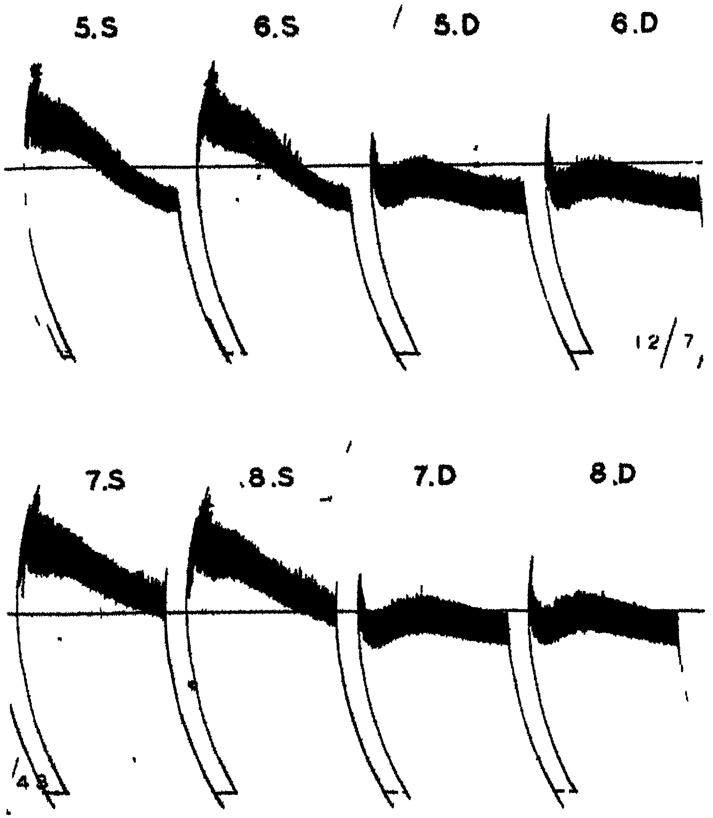


Fig 2. Effects of variations in sponge time on farinograph curves made from sponges and doughs, after sponge fermentation in cabinets and in open troughs (see Table II).

S = sponge, D = dough
5 = 4½-hour sponge; open
6 = 4½-hour sponge, cabinet
7 = 3 hour sponge; open
8 = 3-hour sponge, cabinet

A slight improvement in handling characteristics was noted in machining the cabinet fermented doughs, but no effects on bread quality could be observed; the loaf resulting from the 3-hour cabinet fermentation was no better than the one from the 3-hour open fermentation, and not as good as the 4½-hour loaves, from either open or cabinet fermentation.

The proof time values are significant; the slow proof due to the short sponges shows insufficient fermentation, which was not corrected by the use of the cabinet.

The farinograph curves again showed very definite differences due to the time of sponge fermentation, but no significant difference due to sponge fermentation within the cabinets.

Experiment No. 3. In this experiment, the sponges were mixed at 81.5°F, slightly above the dough room temperature. Table III and Figure 3 show the results obtained. There were no differences between open and cabinet fermentation in this test, either in the sponges, doughs, or the baked bread.

TABLE III

EFFECTS OF CABINET AND OPEN TROUGH FERMENTATION WHEN USING SPONGES MIXED ABOVE THE NORMAL DOUGH ROOM TEMPERATURE

(2% yeast, 60% sponges; dough room, 80°F; sponge, 81.5°F)

	Final sponge temperature		Sponge ferm. loss	Sponge pH	Sponge consist. at 5½ minutes (farinograph units)	Proof time	Loaf volume
	Center	Edge					
	°F	°F	%			*min.*	*cc*
4 hr. sponge; open	93.0	85.75	2.5	4.90	400	69	2,350
4 hr. sponge; cabinet	93.0	85.75	2.5	4.91	410	73	2,375

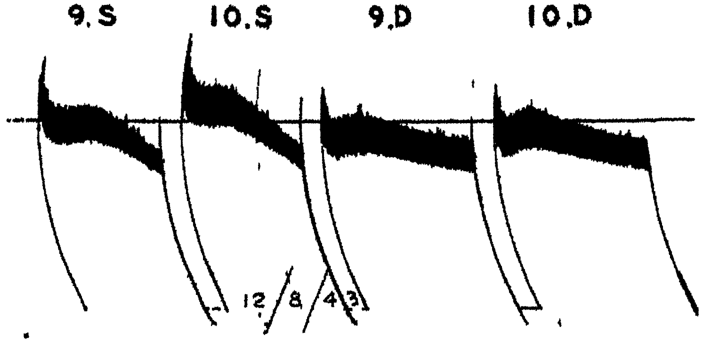


Fig. 3 Farinograph curves made from sponges and doughs after sponge fermentation in cabinets and in open troughs, sponges mixed at temperature above that of the dough room (see Table III).

S = sponge; D = dough
9 = 4-hour sponge; open
10 = 4-hour sponge; cabinet

Experiment No. 4. 60% sponges with 1.5 and 2.0% yeast were used in open and cabinet fermentation, to see whether the lower amount of yeast would show any advantages in the cabinet sponge. Table IV gives the results.

TABLE IV

EFFECTS OF VARIATIONS IN THE AMOUNT OF YEAST WITH CABINET AND OPEN TROUGH FERMENTATION

(4½ hour, 60% sponges; dough room, 80°F; sponge, 77°F)

	Final sponge temperature	Proof time	Loaf volume
	°F	min	
2% yeast; open	90.5	70	2,290
2% yeast; cabinet	90.0	71	2,300
1.5% yeast; open	87.75	80	2,270
1.5% yeast; cabinet	88.5	81	2,255

The differences between open and cabinet fermentation in these tests were negligible, though the cabinet doughs were slightly more mellow in machining. But the differences between 2% and 1.5% yeast were quite distinct in sponge temperature, proof time, and bread score; these were all in favor of 2% yeast, indicating that the use of the cabinet would not permit a reduction in the amount of yeast used.

Experiment No. 5. In this experiment, open trough fermentation in the controlled room at 80°F was compared to open and cabinet fermentation in an uncontrolled room at about 75°F. In another test in the uncontrolled room the top of the trough was covered during the sponge fermentation. The results are shown in Table V.

TABLE V

EFFECTS OF CABINET AND OPEN TROUGH FERMENTATION WHEN USING DIFFERENT DOUGH ROOM CONDITIONS

(2% yeast, 4½ hour, 60% sponges; mixed at 77°F)

	Final sponge temperature	Sponge ferm. loss	Proof time	Loaf volume
	°F	%	min.	cc
Room at 80°F; open	90.0	2.45	69	2,270
Room at 75°F; cabinet	85.0	2.06	74	2,310
Room at 75°F; open	85.5	2.19	74	2,300
Room at 75°F; top covered	85.25	2.06	73	2,290

The differences in sponge temperature, fermentation loss, proof time, and bread score were significant with respect to room temperature, showing that the best fermentation was obtained at 80°F, which also gave the better loaf of bread. Cabinet fermentation at 75°F produced a loaf second in quality, giving better results than open fermentation at 75°F. The covered sponge produced a loaf similar to that of the open trough sponge at 75°F.

It is to be noted that under these uncontrolled conditions at lower room temperature, cabinet fermentation showed some advantage over open trough fermentation in texture and grain, although differences in other respects were negligible.

Use of Thermocouples in Sponge Temperature Measurements

It is reasonable to assume that any benefits derived from cabinet fermentation may be partly due to the insulating effect of the cabinet, thereby minimizing the loss of heat generated during sponge fermentation. In order to get more accurate information on this point, thermocouples were constructed and used for temperature measurement. Figure 4 shows the set-up used for making temperature measurements with thermocouples. Figure 5 shows the positions of the different thermocouples in the trough during sponge fermentation.

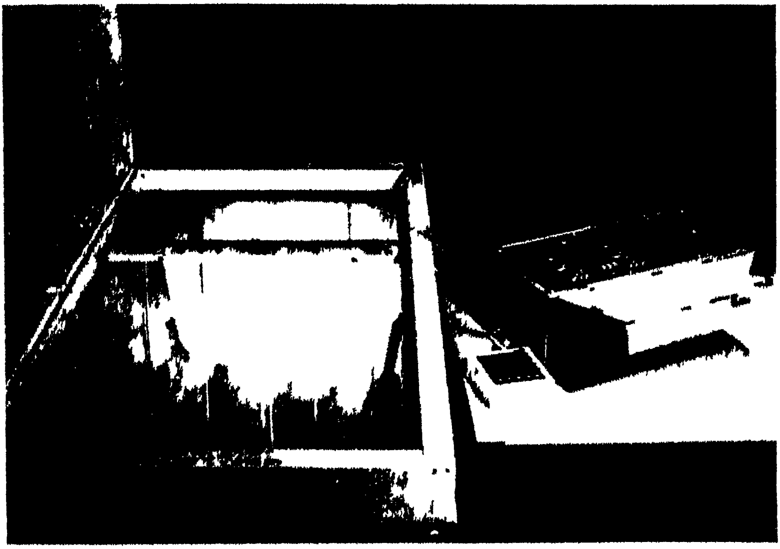


Fig 4 Set up used for measuring temperature changes with thermocouples. Dough trough within cabinet, with removable cover at left, thermocouples in glass tubes mounted in strip of wood resting on edges of trough, wires from thermocouples connected through multiple switch (in center) to potentiometer (at right)

Experiment No. 6. Open and cabinet fermentation were compared in regular sponges in the controlled room at 80°F and 96% humidity; the sponges were mixed at 77°F. The results of temperature measurements at different positions in each sponge are illustrated in Figure 6, showing the temperature changes at two points, post 5 in the center of the sponge, and post 7 at the edge of the sponge in contact with the side of the trough.

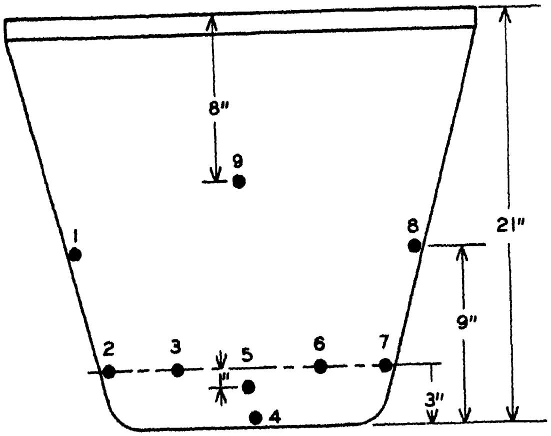


Fig 5 Positions of thermocouples in dough trough Each black circle represents a different thermocouple

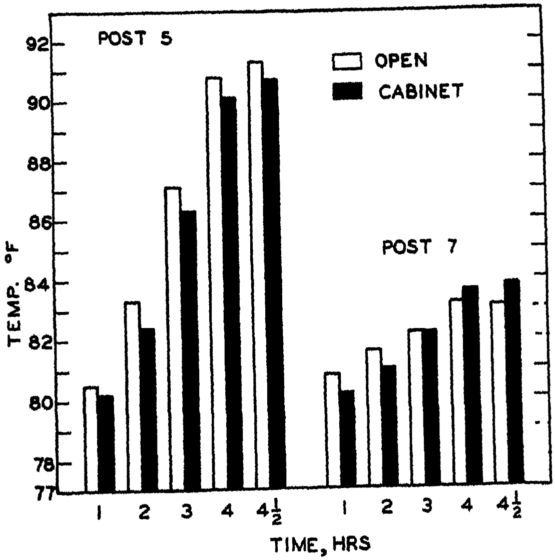


Fig 6 (Experiment 6) Temperatures of sponges fermented in cabinets and in open troughs showing changes in temperature with time, in the center and at the edge of the sponge

Sponge temperatures

Setting temp. 77°F
Dough room temp. 80°F
Yeast 2%, sponge 60%
Half size sponge with trough dam

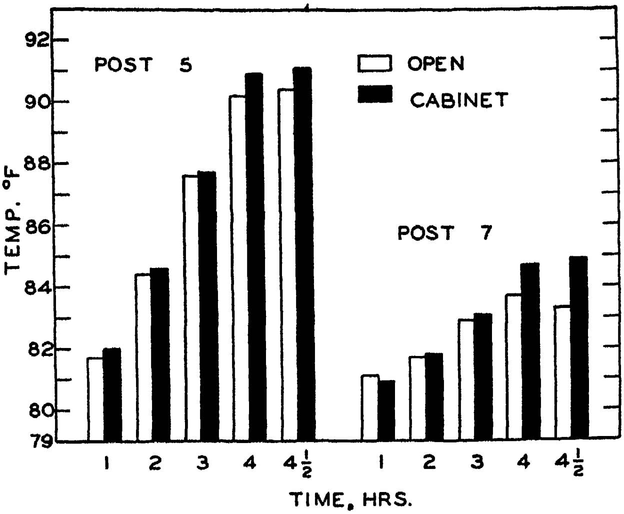


Fig. 7. (Experiment 7.) Temperatures of sponges fermented in cabinets and in open troughs, showing changes in temperature with time, in the center and at the edge of the sponge. Sponges larger than in Experiment No. 6.

Sponge temperatures

Setting temp.	79°F
Dough room temp.	80°F
Yeast 2%, sponge	60%

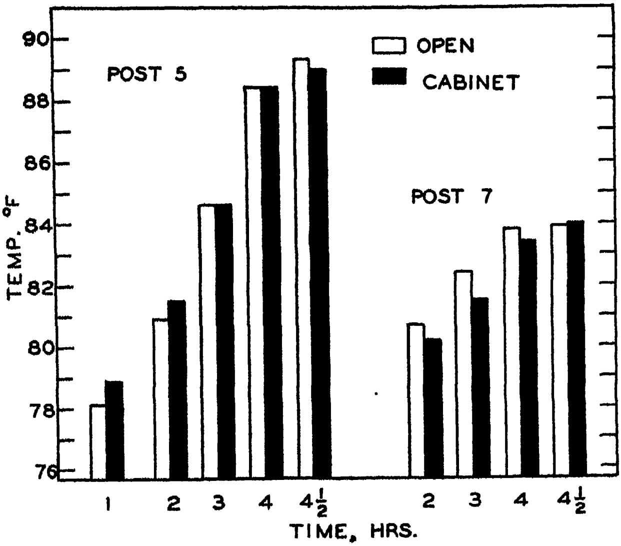


Fig. 8. (Experiment 8.) Temperatures of sponges fermented in cabinets and in open troughs, showing changes in temperature with time, in the center and at the edge of the sponge. Larger sponges with lower initial temperature.

Sponge temperatures

Setting temp.	75.7°F
Dough room temp.	80°F
Yeast 2%, sponge	60%

The temperature differences appear to be insignificant; in the center, the open sponge was about 0.5 degree higher at the end of 4½ hours, while at the edge, the cabinet sponge gained slightly over the open sponge, but was only about 0.5 degree higher after 4½ hours.

The differences in proof time, loaf volume, and bread score were also insignificant, but the cabinet dough was slightly more mellow through the makeup machinery.

Experiment No. 7. In this experiment (Figure 7), double size sponges were used, large enough to fill the trough. The open trough sponge was mixed at 79°F, and the cabinet sponge at 79.5°F; the dough room was at 80°F and 96% humidity. The final temperature in the center was about 0.5 degree higher for the cabinet sponge, but at the edge it was 1.5 degrees higher for the same sponge, partly because of the higher starting temperature. These differences were of little significance, nor were the proof time, volume, and bread score appreciably different. The slightly higher temperature at the edge of the sponge in the cabinet indicates an insulating effect.

Experiment No. 8. This experiment was similar to the preceding one, but the sponge starting temperature was lower; the open trough sponge was mixed at 75.6°F, and the cabinet sponge at 75.7°F.

The temperature readings in this test were almost identical (Figure 8), and differences in fermentation and bread score were negligible; the cabinet dough was slightly more pliable in going through the molder.

Experiment No. 9. Open and cabinet fermentation were now compared in a cool dough room, with the room temperature about 6°F below the starting temperature of the sponge. The results are shown in Table VI.

TABLE VI

Effects of Cabinet and Open Trough Fermentation in a Cool Dough Room (Dough room 71°F, 96% humidity; 2% yeast, 4 hour, 60% sponges; mixed at 77°F)

	Final sponge temperature		Sponge pH	Sponge consist. at 5½ minutes (farinograph units)	Proof time	Loaf volume
	Center	Edge				
	°F	*°F*			*min.*	*cc*
Open	83.8	73.1	4.89	490	77	2,275
Cabinet	85.4	76.4	4.87	430	79	2,350

In this test, cabinet fermentation was definitely superior in almost all respects. Sponge temperature in the cabinet was higher, both in the center and at the edges, although there was a drop in temperature at the edges in both sponges. The cabinet also produced significant differences in farinograph sponge consistency, dough handling in the machines, loaf volume, and bread score. Figures 9 and 10 show the sponge temperature and the farinograph curves for this experiment.

The advantages due to the cabinet in this test appear to result mainly from its insulating effect, as indicated by the temperature

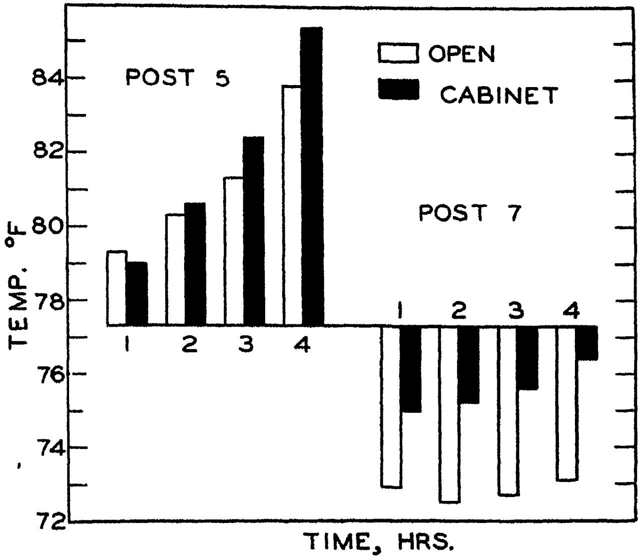


Fig. 9. (Experiment 9.) Temperatures of sponges fermented in cabinets and in open troughs, showing changes in temperature with time, in the center and at the edge of the sponge. Sponges fermented in a cool dough room. Sponge temperature in center lower than in previous experiments, temperature drop at edges.

Sponge temperatures

Setting temp.	77.3°F
Dough room temp.	71°F
Yeast 2%, sponge	60%

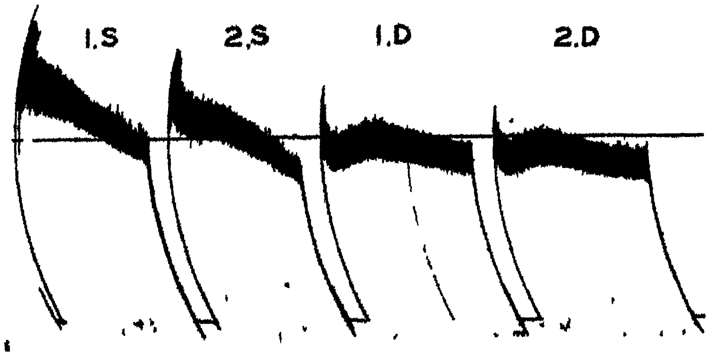


Fig. 10. Farinograph curves made from sponges and doughs after sponge fermentation in cabinets and in open troughs, in a cool dough room.

S = sponge; D = dough
1 = 4-hour sponge; open
2 = 4-hour sponge; cabinet

measurements; this might have been expected, considering the low temperature of the room in which the sponges were fermented.

Discussion

The work presented in this paper indicates that the effectiveness of cabinet fermentation depends on the conditions under which it is used. Since these may vary, one can make no general statement regarding cabinet fermentation, but must specify and define the conditions used, and indicate in just what respects the results were beneficial or otherwise.

The several measurements and observations used as criteria of effectiveness in this work were convincing. All the data, with the exception of slight mellowing effects, showed cabinet fermentation to offer no advantage over open troughs in a controlled dough room at 80°F.[1] Where cabinet fermentation was found advantageous, as in Experiment 9, the insulating effects of the cabinet in a cool room seem to be primarily responsible, and there is no need to resort to involved explanations, or to ascribe mysterious powers to the cabinet in such instances.

The mellowing effects of cabinet fermentation, as indicated by subjective observations, are yet to be explained, though they may be of minor importance from a practical standpoint, if no other changes are involved. It is unlikely that the "blanket of CO_2" theory is valid, since only a small portion of the total sponge surface is exposed, and for a relatively short time, after the sponge break.[2]

Summary

A comparative study of open trough and cabinet fermentation was made under varying conditions of dough room temperature and humidity, sponge temperature, sponge time, and amount of yeast.

The results indicated that cabinet fermentation was of no value under controlled temperature and humidity conditions in which the room temperature was equal to or greater than the temperature at which the sponge was mixed. But when the dough room temperature was much lower than the starting temperature of the sponge, cabinet fermentation showed definite advantages over open trough fermentation.

It is evident that the effectiveness of cabinet fermentation depends on the conditions under which it is used.

---

[1] In a few tests with 100% Kansas flour, not reported in this paper, the results were very similar to those obtained with the flour blend used in these experiments.

[2] A few experiments in which carbon dioxide was deliberately admitted into the cabinet above the sponge failed to show any difference not previously observed without its addition.

AMINO ACIDS IN CORN GRAIN FROM SEVERAL SINGLE CROSS HYBRIDS[1, 2]

D. M. Doty, Merlin S. Bergdoll, Harold A. Nash, and A. M. Brunson[3]

Departments of Agricultural Chemistry and Botany and Plant Pathology, Purdue University Agricultural Experiment Station, Lafayette, Indiana

(Received for publication December 10, 1945)

It is quite generally recognized that the genetic constitution of a plant may affect its chemical composition, but there is very little published work showing the effect of genetic constitution of the plant on the chemical composition of corn grain. The widespread use of commercial corn hybrids makes it highly desirable to obtain this type of information since the feeding value of corn is dependent, in part at least, on its chemical composition.

The work reported in this paper was conducted to determine whether or not grain from different single crosses among inbred lines of corn varies in its content of protein and certain nutritionally important amino acids. The results indicate that the amounts of tyrosine, tryptophane, cystine, arginine, and histidine present in corn grain are related to the genetic constitution of the plant.

It is known that the protein content of corn grain is variable and may be affected by environmental factors such as soil type, fertilizer treatment, and moisture supply (Doty *et al.*, 1943; Fraps, 1931; Greaves and Nelson, 1925; Whitson *et al.*, 1902; Widstoe, 1903). Data presented in the fifty-first annual report of the Illinois Agricultural Experiment Station (1942) show also that the percentage of protein in corn grain can be increased or decreased by continuous ear-to-row selection.

A few reports have been published on the amino acid composition of the complete protein fraction from corn grain. Csonka (1939) found that Boone County White corn grown in Virginia contained practically the same amounts of cystine, tryptophane, tyrosine, arginine, histidine, and lysine as Black Yellow Dent corn grown in Iowa. Hamilton *et al* (1921) found that amide nitrogen made up 11.94% of the total nitrogen of corn grain; cystine nitrogen made up 1.07%, arginine nitrogen 8.73%, histidine nitrogen 4.83%, and lysine nitrogen 2.20%. Morris (1934) obtained similar values for flaked maize, except that amide nitrogen and histidine nitrogen were lower.

---

[1] Journal Paper No. 221 of the Purdue University Agricultural Experiment Station. Herman Frasch Foundation for Agricultural Research, Paper No. 227.

[2] In cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Senior Agronomist, U. S. Department of Agriculture.

Zein, a prolamine which makes up 25 to 60% of the total protein of corn grain, has been carefully analyzed for its amino acid content by numerous investigators. This work has been summarized by Hoffman (1925) and Mitchell (1929).

Csonka (1932) and Jones and Csonka (1928) found that α-glutelin from corn contained more lysine, more tryptophane, more arginine, and more histidine than zein.

May and Rose (1922) found that maize gluten contained 1.08% tryptophane, while Matsuyama and Mori (1923) reported that corn protein contained only a trace of tryptophane.

These investigations indicate that the amino acid composition of the complete protein fraction of corn grain may depend upon the relative amounts of the various types of protein present in corn. Osborne and Mendel (1914) found that 22% of the total corn protein was soluble in 10% potassium chloride solution, 41% was soluble in 90% ethanol, 31% was soluble in 0.2% potassium hydroxide solution, and 6% was insoluble or lost. Spitzer, Carr, and Epple (1919) found 5.27% of the nitrogen of corn present as amide nitrogen, 21.61% soluble in 10% sodium chloride solution, 29.41% soluble in 90% ethanol, and 42.81% as glutelin nitrogen. Later, Showalter and Carr (1922) found that greater percentages of the protein of a high-nitrogen corn sample were soluble in 90% ethanol and 10% sodium chloride than of the protein of a low-nitrogen corn sample. More recent work by Zeleny (1935) indicates that the relative proportions of the various proteins in corn grain may be dependent upon the stage of maturity of the corn.

Materials and Methods

The samples of corn grain used in this study were obtained from corn grown on experimental plots on the Soils and Crops Farm near Lafayette, Indiana. In 1939, the samples used were composite samples from triplicate plots. In 1940 and 1941 the samples were taken from single plots. All samples were harvested at maturity, artificially dried, and shelled. The shelled corn was stored at −5°C until ground for analysis.

The methods used for analysis were those described by Doty (1941) with a few exceptions. It was found that in some samples tryptophane could not be determined satisfactorily in an alkali hydrolyzate, hence it was necessary to use the following procedure for the determination of tryptophane.

Place the residue from the saliva digestion (Doty, 1941) of 1 g of dry fat-free corn in a 50 ml beaker. Add 1.0 ml of 2.5% p-dimethylaminobenzaldehyde solution and 0.5 ml of 0.15% sodium nitrate solution. Stir for 2 minutes, add 25 ml of concentrated hydrochloric acid, and stir. Cover the beaker and allow to stand for 18 hours at room temperature. Dilute the deep blue solution with 10 ml of water, add a pinch of asbestos fiber, and allow to stand for 1 hour at room temperature. Transfer the solution to a 100 ml volumetric flask, add 25 ml of concentrated hydrochloric acid, and make to volume with water. Mix and filter through Whatman No. 42 filter paper. Use a portion of the filtrate to determine the transmission value in the KWSZ photometer. Determine the amount of tryptophane present by reading the transmission value from a standard curve prepared by using known amounts of tryptophane in the procedure outlined above.

It was also found that some batches of Norit A, even after washing with acid and alkali, could not be used for decolorizing the alkaline hydrolyzates because tyrosine was adsorbed to a slight extent. Therefore, the alkaline hydrolyzates were decolorized by shaking about 18 ml of solution in a test tube with approximately 0.3 g of talc and filtering immediately through Whatman No. 42 filter paper.

Histidine determinations on the 1940 and 1941 samples were carried out by the sulfanilic acid method described by Macpherson (1942) except that the color developed was measured in the KWSZ photometer equipped with Corning filter no. 406 and Jena filter GG 11. Several of the samples were analyzed both by this method and the method described by Doty (1941), and it was found that the results agreed perfectly.

Protein fractionation studies were carried out as follows: Extract dry corn, ground to pass an 80 mesh sieve, with dry ethyl ether in a Soxhlet extraction apparatus for 24 hours. Remove the ether from the residue, and shake up 2.5 g of the dry residue with 100 ml of distilled water in a 250 ml centrifuge tube. Let stand for several minutes at room temperature with occasional shaking. Centrifuge, pour off the supernatant liquid, add 75 ml of water, and repeat the extraction. Repeat the water extraction a third time. Make the combined water extracts to volume and use an aliquot for total nitrogen determination. Extract the residue with 5% sodium chloride solution for 1 hour at room temperature with occasional shaking. Centrifuge and repeat the extraction twice. Add 100 ml of 80% ethanol to the residue, mix, and allow to stand overnight. Centrifuge and re-extract twice with 80% ethanol for periods of 1 hour each. Finally extract three times with 0.2% sodium hydroxide solution. Allow the suspension to stand at room temperature for 1 hour with occasional shaking for each extraction. In each case use a total volume of 250 ml of extracting solution, make the combined extracts to volume, and use an aliquot for total nitrogen determination.

In all cases where analysis of variance was made of the data, the standard error of the mean difference was calculated and used to derive the corresponding *t* value, the significance of which was ascertained from Snedecor's (1938) tables. Throughout the discussion, differences having odds of 19:1 or more against their being due to random variation are termed "significant" and differences having odds of 99:1 or more against their being due to random variation are termed "very significant."

Experimental Results and Discussion

Samples of corn grain from 28 single crosses grown in 1939 were analyzed for total protein, cystine, arginine, histidine, tryptophane, and tyrosine. The results (Table I) show that the protein content of the hybrids varied considerably and that the amino acid distribution of the protein fraction differed with the various samples. There was some indication that protein content and amino acid content of the protein were related to genetic constitution. For example, all hybrids having Mid 1 or Bl 13092 as one parent contained relatively large percentages of protein, while hybrids having 540 or Mo940 as one parent contained relatively small percentages of protein. Also, in 1939, hybrids having Mo940 or 540 as one parent contained less cystine per gram of nitrogen than hybrids of other parentage, while hybrids involving Mid 1 contained relatively large amounts of cystine. Hybrids having Bl 13092 or LE 43 as one parent contained much smaller amounts of arginine than did the remainder of the samples.

The data on the samples grown in 1939 can indicate only trends as regards the influence of genetic constitution on amino acid distribution of the protein, because the effect of only one parent, irrespective of the other parent, can be evaluated. Moreover, the effect of seasonal variation cannot be determined on the basis of one year's results; and seasonal variations are important as shown by the fact that hybrids of the same genetic constitution grown in 1939 and in 1941 varied greatly in total protein and amino acid content (Table I).

To evaluate more closely the effect of genetic constitution on amino acid content, two series of single crosses were grown in 1940 and 1941, and amino acids of the protein fraction from all samples determined. The midseason series consisted of all possible combinations of inbred lines L317, Hy, 38–11, WF9, 187–2, and R4; the late series consisted of all possible combinations of inbred lines Mo940, Hy, 38–11, L317, Mo824, and K4.

The results of the analyses show that there was considerable variation in the amino acid content of the hybrids (Tables II and III). In the first place, season apparently affected the composition to a con-

TABLE I

Total Protein and Amino Acid Content of Dry Corn Grain from Miscellaneous Single Crosses

Hybrid pedigree designation	Year grown	Total protein	Amino acid content per gram of nitrogen				
			Cystine	Arginine	Histidine	Tryptophane	Tyrosine
		%	*mg*	*mg*	*mg*	*mg*	*mg*
Mid 1 × YS79	1939	9.4	71	202	132	33.9	372
Mid 1 × 38-11	1939	9.8	81	184	156	31.6	405
Mid 1 × 38-11	1941	10.7	80	215	127	33.6	399
Mid 1 × C9	1939	9.5	69	218	135	33.4	385
Mid 1 × C9	1941	10.5	83	220	133	33.3	394
Mid 1 × KD102	1939	9.9	71	197	147	32.6	387
Mid 1 × Mo824	1939	10.6	79	227	160	31.4	402
Mid 1 × Mo940	1939	8.8	80	234	157	35.0	427
Mid 1 × 540	1939	9.0	71	217	125	31.2	353
Mid 1 × 540	1941	10.0	74	215	124	33.7	394
Mid 1 × K4	1941	10.6	90	212	117	33.6	396
Mo940 × Hy31	1939	8.1	74	186	123	31.3	438
Mo940 × YS79	1939	8.4	56	220	117	34.9	414
Mo940 × 38-11	1939	8.8	52	199	120	35.5	425
Mo940 × C9	1939	7.5	69	189	123	40.5	412
Mo940 × C9	1941	8.9	76	241	120	38.2	434
Mo940 × KD102	1939	7.9	59	185	129	36.5	410
Mo940 × Mo824	1939	8.6	62	182	117	33.5	415
Mo940 × 540	1939	8.4	71	214	138	35.5	398
Mo940 × 540	1941	7.5	77	264	125	45.0	459
540 × Hy31	1939	8.9	55	223	147	33.7	405
540 × YS79	1939	7.9	55	221	116	35.7	357
540 × C9	1939	7.9	72	183	153	39.5	400
540 × C9	1941	8.1	79	260	132	44.2	428
540 × 38-11	1939	8.9	68	216	130	31.8	405
540 × 38-11	1941	8.9	69	253	133	40.5	410
540 × KD102	1939	8.0	68	200	137	36.5	401
540 × Mo824	1939	8.4	65	211	131	35.5	434
540 × K4	1941	9.3	83	253	129	40.7	444
LE43 × 38-11	1939	9.1	78	132	140	30.7	380
LE43 × WF9	1939	8.4	74	144	118	31.8	367
LE43 × 540	1939	8.6	58	140	156	32.7	385
LE43 × P8	1939	9.6	66	135	160	29.0	351
Bl 13092 × R4	1939	9.8	63	131	152	29.6	367
Bl 13092 × R4	1941	9.4	81	239	131	38.2	388
Bl 13092 × 38-11	1939	10.2	76	150	142	29.4	375
Bl 13092 × 540	1939	9.8	51	139	129	29.9	360
Bl 13092 × 540	1941	10.4	81	215	131	32.6	420
Bl 13092 × Hy	1941	10.2	76	231	130	36.3	411
Bl 13092 × WF9	1941	11.9	88	233	146	33.5	367
C9 × K4	1941	10.5	78	230	123	36.8	398
C9 × 38-11	1941	9.7	78	223	120	36.7	407
C9 × Mo824	1941	10.0	74	230	121	36.9	403

All figures given are averages from at least two separate determinations which did not vary from the average by more than 5%.

TABLE II

TOTAL PROTEIN AND AMINO ACID CONTENT OF DRY CORN GRAIN FROM A SERIES OF MIDSEASON SINGLE CROSSES

Hybrid pedigree designation	Year grown	Total protein	Amino acid content per gram of nitrogen				
			Cystine	Arginine	Histidine	Tryptophane	Tyrosine
		%	mg	mg	mg	mg	mg
L317 × Hy	1940	9.6	81	208	109	31.2	396
L317 × Hy	1941	9.8	77	210	123	33.0	384
L317 × 38–11	1940	10.4	78	217	113	26.5	374
L317 × 38–11	1941	10.5	79	202	126	30.9	373
L317 × WF9	1940	10.5	79	208	125	30.1	369
L317 × WF9	1941	8.5	81	243	135	36.0	378
L317 × 187–2	1940	10.4	71	194	112	30.0	368
L317 × 187–2	1941	9.4	81	258	127	34.6	361
L317 × R4	1940	9.4	75	211	105	32.7	387
L317 × R4	1941	9.0	79	228	127	30.0	358
Hy × 38–11	1940	9.8	74	218	120	28.7	427
Hy × 38–11	1941	9.8	73	223	119	33.0	394
Hy × WF9	1940	9.8	63	153	111	31.8	395
Hy × WF9	1941	9.1	88	234	128	36.5	377
Hy × 187–2	1940	9.3	74	215	125	33.5	428
Hy × 187–2	1941	9.5	79	230	124	33.6	395
Hy × R4	1940	9.2	76	218	105	36.3	422
Hy × R4	1941	9.8	80	223	123	35.6	384
38–11 × WF9	1940	10.9	76	200	125	26.3	377
38–11 × WF9	1941	10.4	83	217	126	32.6	366
38–11 × 187–2	1940	10.5	67	196	110	25.6	357
38–11 × 187–2	1941	10.0	74	206	117	31.8	385
38–11 × R4	1940	10.5	77	190	112	29.8	405
38–11 × R4	1941	11.0	80	250	124	31.8	379
WF9 × 187–2	1940	10.1	75	210	131	31.4	389
WF9 × 187–2	1941	10.4	83	223	132	36.8	356
WF9 × R4	1940	10.1	73	197	113	29.8	370
WF9 × R4	1941	9.3	83	235	130	35.5	351
187–2 × R4	1940	9.2	80	218	114	32.6	394
187–2 × R4	1941	9.9	75	221	133	35.5	367

See footnote, Table I.

siderable extent. Analysis of variance of the data showed that for the series of midseason hybrids, the average values for cystine, arginine, histidine, and tryptophane were all very significantly higher in 1941 than they were in 1940. For the late series, the average histidine

TABLE III

TOTAL PROTEIN AND AMINO ACID CONTENT OF DRY CORN GRAIN FROM A SERIES OF LATE YELLOW SINGLE CROSSES

Hybrid pedigree designation	Year grown	Total protein	Amino acid content per gram of nitrogen				
			Cystine	Arginine	Histidine	Tryptophane	Tyrosine
		%	mg	mg	mg	mg	mg
Mo940 × Hy	1940	9.6	70	210	119	29.9	396
Mo940 × Hy	1941	11.4	74	229	124	31.2	393
Mo940 × 38-11	1940	9.9	78	206	122	29.7	393
Mo940 × 38-11	1941	11.4	76	218	124	31.6	383
Mo940 × L317	1940	8.9	88	211	111	30.3	444
Mo940 × L317	1941	10.0	83	238	138	32.5	381
Mo940 × Mo824	1940	10.5	66	179	102	26.7	357
Mo940 × Mo824	1941	11.0	72	221	116	31.3	379
Mo940 × K4	1940	10.1	75	192	109	29.6	395
Mo940 × K4	1941	11.1	78	236	121	33.1	378
Hy × 38-11	1940	9.7	72	213	113	29.1	393
Hy × 38-11	1941	10.3	70	224	112	33.3	398
Hy × L317	1940	8.5	77	206	107	33.8	427
Hy × L317	1941	9.3	76	222	110	34.2	398
Hy × Mo824	1940	9.9	70	197	102	28.4	367
Hy × Mo824	1941	10.0	75	225	111	33.7	400
Hy × K4	1940	10.2	74	190	101	28.2	410
Hy × K4	1941	10.8	73	220	109	32.4	401
38-11 × L317	1940	9.4	83	220	142	29.7	391
38-11 × L317	1941	8.7	82	230	129	35.2	425
38-11 × Mo824	1940	10.2	72	190	107	26.7	368
38-11 × Mo824	1941	10.3	78	218	115	31.5	404
38-11 × K4	1940	10.2	80	208	116	26.4	393
38-11 × K4	1941	10.0	85	231	134	35.0	437
L317 × Mo824	1940	10.1	80	179	110	30.2	383
L317 × Mo824	1941	10.4	77	217	116	31.9	416
L317 × K4	1940	10.0	81	200	107	29.3	405
L317 × K4	1941	8.1	78	238	121	36.1	430
Mo824 × K4	1940	11.1	69	168	90	26.4	366
Mo824 × K4	1941	8.7	83	236	129	35.3	425

See footnote, Table I

content was significantly higher, and the average values for arginine and tryptophane were very significantly higher in 1941 than in 1940.

From inspection of the data in Tables II and III it appears that in some cases the amino acid content of the protein of the hybrid was related to the genetic constitution. For example, among the mid-season crosses, all hybrids involving 38–11 were somewhat lower in tryptophane than were hybrids with other parentage. In order to determine whether or not such differences were significant, the data from each series were separated into groups of hybrids with one common parent and the data for each group compared to that for a second group with a different common parent. For example, the group consisting of L317 × 38–11, L317 × WF9, L317 × 187–2, and L317 × R4 was compared with the group consisting of Hy × 38-11, Hy × WF9, Hy × 187–2, and Hy × R4. In this way the relative effects of L317 and Hy on the amino acid content of the hybrids could be ascertained.

TABLE IV

SIGNIFICANT DIFFERENCES IN THE PROTEIN AND AMINO ACID CONTENT OF GRAIN FROM MIDSEASON CROSSES AS RELATED TO THE INBREDS INVOLVED

Protein	Hybrids involving 38–11 higher than L317, Hy,* 187-2,* or R4* crosses
Tryptophane	Hybrids involving Hy higher than L317,* WF9,* 187-2,* R4,* or 38-11 crosses
Tyrosine	Hybrids involving Hy,* WF9,* 187-2,* or R4* higher than 38-11 crosses
Histidine	Hybrids involving WF9 higher than L317, Hy, 38-11, or R4 crosses Hybrids involving 187–2 higher than Hy crosses
Cystine	No significant differences
Arginine	No significant differences

* Very significant (>99:1).

TABLE V

SIGNIFICANT DIFFERENCES IN THE PROTEIN AND AMINO ACID CONTENT OF GRAIN FROM LATE YELLOW SINGLE CROSSES AS RELATED TO THE INBREDS INVOLVED

Protein	Hybrids involving Hy, Mo940,* 38-11, Mo824,* or K4 higher than L317 crosses
Tryptophane	Hybrids involving L317 higher than Mo940 or Hy crosses
Tyrosine	Hybrids involving L317 higher than Mo940* or Mo824 crosses Hybrids involving K4 higher than Mo824 crosses
Cystine	Hybrids involving L317 higher than Mo940,* Mo824,* or Hy* crosses Hybrids involving K4 higher than Hy crosses Hybrids involving 38–11 higher than Hy* crosses
Arginine	Hybrids involving K4,* 38–11, Mo940, or Hy higher than Mo824 crosses
Histidine	Hybrids involving 38–11 higher than Hy crosses Hybrids involving Mo940 higher than Mo824 crosses

* Very significant (>99:1).

Analysis of variance on data organized in this way showed that in many cases the amino acid content of the hybrids was significantly affected by the parentage of the hybrids (Tables IV and V). These effects were actually on the protein and were not necessarily related to the total protein content of the corn grain, since the amino acid contents were expressed as the amounts of amino acids per unit of nitrogen.

The data and the discussion just completed indicate that the amino acid distribution in corn protein is heritable, and may be controlled, to some extent at least, by choosing the inbreds which are used to develop a hybrid. Since the amino acid distribution in the protein was shown to be different in different hybrids, then the physico-chemical behavior of grain protein from different hybrids might be expected to vary. To test this, the solubility of the nitrogen in the grain from two hybrids grown in 1940 was determined. The results (Table VI) show that the solubility characteristics of the protein from the two samples were distinctly different. The grain from L317 X 38-11 contained more alkali-soluble and less alcohol-soluble nitrogen than did grain from Mo940 X Mo824. This is in harmony with the fact that L317 X 38-11 contained greater proportions of cystine, arginine, histidine, tryptophane, and tyrosine in its protein than did Mo940 X Mo824, since reports by Csonka (1932), Hoffman (1925), Jones and Csonka (1928), May and Rose (1922), and Mitchell (1929) show that the glutelin (dilute alkali-soluble) fraction of corn protein contains greater proportions of these amino acids than does the zein (alcohol-soluble) fraction. If the view of seed proteins as elaborated by Gortner (1938) is accepted, then the results discussed above must

TABLE VI

AMINO ACID COMPOSITION AND PROTEIN-SOLUBILITY OF GRAIN FROM TWO SINGLE CROSS HYBRIDS GROWN IN 1940

	L317 X38-11	Mo940 XMo824
Total protein (% on dry wt. basis)	9.4	10.5
Cystine (mg per g N)	83	66
Arginine (mg per g N)	220	179
Histidine (mg per g N)	142	102
Tryptophane (mg per g N)	29.7	26.7
Tyrosine (mg per g N)	391	357
Nitrogen sol. in H_2O (% of total N)	10.8	12.6
Nitrogen sol. in 5% NaCl (% of total N)	12.0	12.8
Nitrogen sol. in 80% C_2H_5OH (% of total N)	20.5	23.3
Nitrogen sol. in 0.2% NaOH (% of total N)	48.4	43.4
Nitrogen of insoluble residue (% of total N)	12.7	10.5
Nitrogen recovered (% of total N)	104.4	102.6

be interpreted to mean that the protein complex of the two hybrids was distinctly different.

In general, the amino acid content of corn grain as reported here agrees very well with results published by Csonka (1939), Hamilton *et al.* (1921), and Morris (1934).

To our knowledge, it has been shown for the first time that the amounts of certain amino acids in corn protein are affected, to some extent at least, by the genetic constitution of the plant. The occurrence of several highly significant differences in amino acid content among single crosses from a few random inbred lines indicates that in all probability a wider range in composition could be found if a larger number of inbred lines were studied similarly. Therefore, it might be possible by careful breeding to develop a hybrid with a high proportion of nutritionally essential amino acids in its protein.

Summary

The grain from 28 corn single crosses grown in 1939 was analyzed for total protein, tryptophane, tyrosine, cystine, arginine, and histidine. There was some indication that the amount of the various amino acids in the corn protein was related to the genetic constitution of the hybrids.

In many cases the amino acid content of the corn protein was related to the inbred lines involved in single cross hybrids grown in 1940 and 1941.

The physico-chemical nature of the protein in the grain from two single cross hybrids was distinctly different as shown by the fact that the sample which contained the larger amounts of cystine, arginine, histidine, tryptophane, and tyrosine also contained a larger percentage of alkali-soluble nitrogen and a smaller percentage of alcohol-soluble nitrogen.

Literature Cited

Csonka, F. A.
1932 Studies on glutelins. VII. Cystine, tryptophane, and tyrosine content of glutelins. J. Biol. Chem. 97: 281 286.
1939 Amino acids in the corn kernel. J. Agr. Research 59: 765–768.
Doty, D. M.
1941 Methods for estimation of some amino acids in corn grain. Ind. Eng. Chem. (Anal. Ed.) 13: 169-172.
——, Bergdoll, M. S., and Miles, S. R.
1943 The chemical composition of commercial hybrid and open-pollinated varieties of dent corn and its relation to soil, season, and degree of maturity. (A preliminary report.) Cereal Chem. 20: 113–120.
Fraps, G. S.
1931 Variations in vitamin A and chemical composition of corn. Texas Agr. Expt. Sta. Bull. 422.
Gortner, R. A.
1938 Outlines of Biochemistry (2nd ed.). John Wiley and Sons, New York, N. Y.

Greaves, J. E., and Nelson, D. H.
1925 The influence of irrigation water and manure on the composition of the corn kernel. J. Agr. Research **31**: 183–189.
Hamilton, T. S., Nevens, W. B., and Grindley, H. S.
1921 The quantitative determination of amino-acids of feeds. J. Biol. Chem. **48**: 249 272.
Hoffman, W. F., and Gortner, R. A.
1925 Physico-chemical studies on proteins: The prolamines, their chemical composition in relation to acid and alkali binding. Colloid Symposium Monograph, Vol. II, pp. 209–368. Chemical Catalogue Co., New York, N. Y.
Illinois Agr. Expt. Sta., 51st annual report
1942 High-low chemical strains well established in white corn. pp. 47–48.
Jones, D. B., and Csonka, F. A.
1928 Studies on glutelins. IV. The glutelins of corn (*Zea mays*). J. Biol. Chem. **78**: 289-292.
Macpherson, H. T.
1942 Modified procedures for the colorimetric estimation of arginine and histidine. Biochem. J. **36**: 59–63.
Matsuyama, Y., and Mori, T.
1923 Quantities of tryptophan in various proteins. J. Chem. Soc. Japan **44**: 377 381; Chem. Abs. **17**: 2718.
May, C. E., and Rose, E. R.
1922 The tryptophane content of some proteins. J. Biol. Chem. **54**: 213–216.
Mitchell, H. H.
1929 The Biochemistry of the Amino Acids. pp. 180–181. Chemical Catalogue Co., New York, N. Y.
Morris, S.
1934 The nutritive value of proteins for milk production. III. The determination of the amino acids of various feeds. J. Dairy Research **5**: 108-112.
Osborne, T. B., and Mendel, L. B.
1914 Nutritive properties of proteins of the maize kernel. J. Biol. Chem. **18**: 1–16.
Showalter, M. F., and Carr, R. H.
1922 Characteristic proteins in high- and low-protein corn. J. Am. Chem. Soc. **44**: 2019-2023.
Snedecor, G. W.
1938 Statistical Methods Applied to Experiments in Agriculture and Biology. Collegiate Press, Ames, Iowa.
Spitzer, G., Carr, R. H., and Epple, W. F.
1919 Soft corn Its chemical composition and nitrogen distribution. J. Am. Chem. Soc. **41**: 1212 1221.
Whitson, A. R., Wells, F. J., and Vivian, A.
1902 Influence of the soil on the protein content of crops. Wisc. Agr. Expt. Sta., 19th Ann. Rept., pp. 192 209.
Widstoe, J. A.
1903 The influence of soil moisture upon the chemical composition of certain plant parts. J. Am. Chem. Soc. **25**: 1234-1243.
Zeleny, L.
1935 The distribution of nitrogen in the seed of *Zea mays* at different stages of maturity. Cereal Chem. **12**: 536–542.

AN AUTOMATIC DEVICE FOR THE MEASUREMENT OF GAS PRODUCTION AND GAS RETENTION IN DOUGHS [1]

EARL B. WORKING [2] and EMERY C. SWANSON

Department of Milling Industry,
Kansas State College, Manhattan, Kansas

(Received for publication September 5, 1945)

Numerous published papers dealing with the measurement and significance of gas production and gas retention in fermenting doughs have been reviewed by Elion (1940). The gas produced may be measured volumetrically, as by the procedure of Bailey and Johnson (1924). Similar devices have been described by Jørgensen (1931), Markley and Bailey (1932), Heald (1932), Sherwood, Hildebrand, and McClellan (1940), and Hullett (1941). Gas production has been measured manometrically by Blish, Sandstedt, and Astleford (1932) and by the widely used apparatus of Sandstedt and Blish (1934) and its modification (Malloch, 1939). An automatic, manometric apparatus has been described by Miller, Edgar, and Whiteside (1943). A gravimetric procedure has been described by Elion (1939). Eva, Geddes, and Frisell (1937) compared several methods of measuring gassing power and found the correlation between them to be of very high degree.

The apparatus described in this paper embodies the principles used in the Chefaro balance (Elion, 1939) except that, instead of using weight as a method of measurement, the pressure of the gas produced during fermentation in a sealed jar of known and constant volume is automatically recorded by electric sparks which burn holes in a chart paper. Many of the mechanical principles used were suggested by Mr. C. F. Buck who constructed the apparatus used by Heald (1932).

Description of Apparatus

The apparatus, illustrated in Figure 1, consists principally of a constant temperature water bath, two revolving aluminum drums holding the graph paper on which the data are recorded, four mercury manometers which activate a stylus, glass jars weighed down so as to remain under water during the course of the experiment, and an electrical circuit for producing the sparks.

The water bath, size 18″ × 12″ × 7″, is large enough to hold four jars, a mechanical stirrer to keep the water agitated, a thermometer, and a thermoregulator. The temperature of the bath is maintained at 30°C during measurements.

---

[1] Contribution No. 115, Department of Milling Industry.

[2] Formerly Professor in Milling Industry at Kansas State College, Manhattan, Kansas, now consulting chemist at Palo Alto, California.

The jars are wide-mouth, 4 inches high, and 3¾ inches in diameter outside measurement, and each of 500 ml volume. Inside each jar is placed a glass triangle (bent glass tubing) to support a watch glass upon which the dough is placed. During operation, the jars are sealed gastight with rubber-gasketed brass lids which are bolted to a brass platform. This can be raised when placing and removing doughs. On each brass lid is a release valve and also an outlet which is coupled with a mercury manometer by means of thick-walled rubber

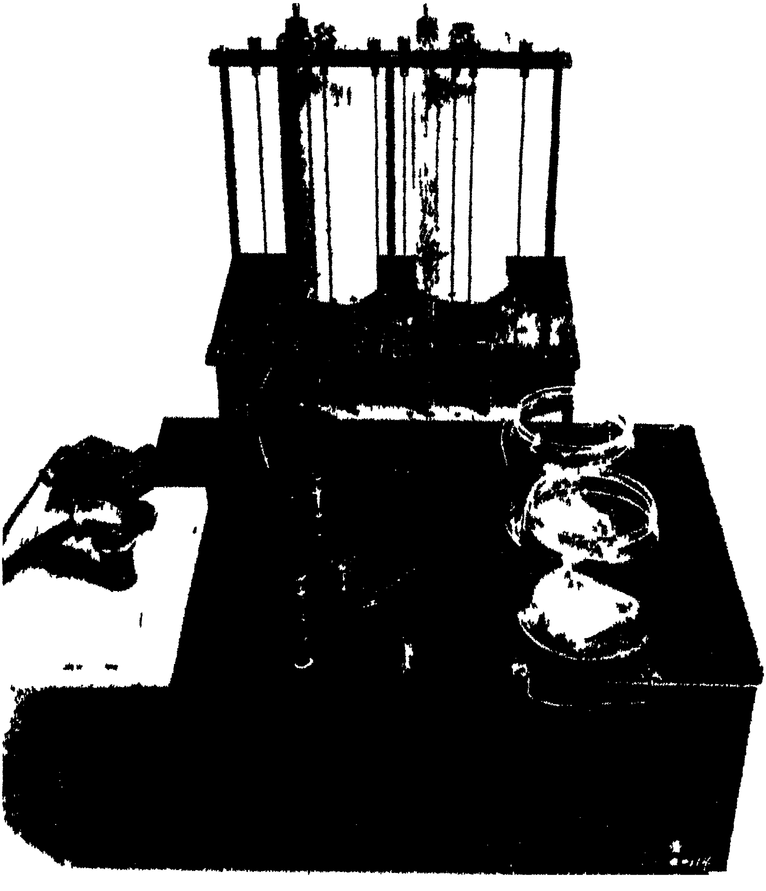


Fig 1 Original automatic recording gasometer at rest

tubing. The pressure in the jars is produced by the expansion of the fermenting dough and also by the carbon dioxide which escapes from the dough, unless the latter is absorbed by alkali. A 23% sodium chloride solution (25 ml) is used for measuring gas production and a 23% potassium hydroxide (or other alkali such as sodium hydroxide) solution is placed in the jar for measuring gas retention.

As the pressure is increased, a column of mercury rises in a 10 mm glass tube whose lower end is below the surface of the mercury container in a small sealed bottle which acts as a reservoir and is directly connected to the fermentation jars by heavy rubber tubing. This vertically raises the floating aluminum-cork stylus. The height of the point of the stylus measures the pressure within the connected jar. When no pressure activates the stylus, the recording point is at rest at zero position. At all times, regardless of pressure, the point of the stylus is about one-eighth of an inch from the revolving aluminum drum. The guiding of this point is by means of the vertical rods shown in front of the drums.

The diameter of the revolving drums is such that the usual cross-section graph paper (cross-ruled in 1/20 of an inch) can be wrapped around once. Each drum is rotated by a clockwork gear mechanism driven by an electric clock motor of one rpm. Each drum has a circumferential speed of one inch per hour and will rotate for 8 hours and then automatically shut off. Any shorter period can be used by properly adjusting the drums.

The recording of the pressures in the jars is by sparks from an A.C.-operated induction coil. The duration of sparking is controlled by adjusting the position of a contact switch on the clock motor shaft. The circuit for a particular stylus closes and a spark passes from the point of the stylus across the gap and into the aluminum drum. This burns a small hole through the graph paper. Each stylus sparks once every 4 minutes, leaving 15 dots per inch of horizontal travel, thus outlining a continuous graph of the pressure within the connected jar from the start of operation until the machine automatically stops. The gas retention pressure is recorded in a similar manner.

Since the volume within the jar is constant, any increase in the internal pressure is directly proportional to the volume of the gas produced (or retained). The volume of gas produced or retained is therefore determined from the pressure readings on the graph by means of an equation based on Boyle's law. For the particular volume of the jars used, an increase in pressure of one inch of mercury equals 15.88 ml of gas.

The apparatus has a capacity of four samples, the dough having the same consistency as that used for bread. Four samples may be run for gas production only or gas retention only, or two samples for gas production and two for gas retention, depending on whether a solution of sodium chloride or of sodium hydroxide is placed under the watch glasses.

A pair of typical curves is given in Figure 2, the upper showing total production and the lower total retention. The pressure is measured on the Y-axis and the time of fermentation on the X-axis.

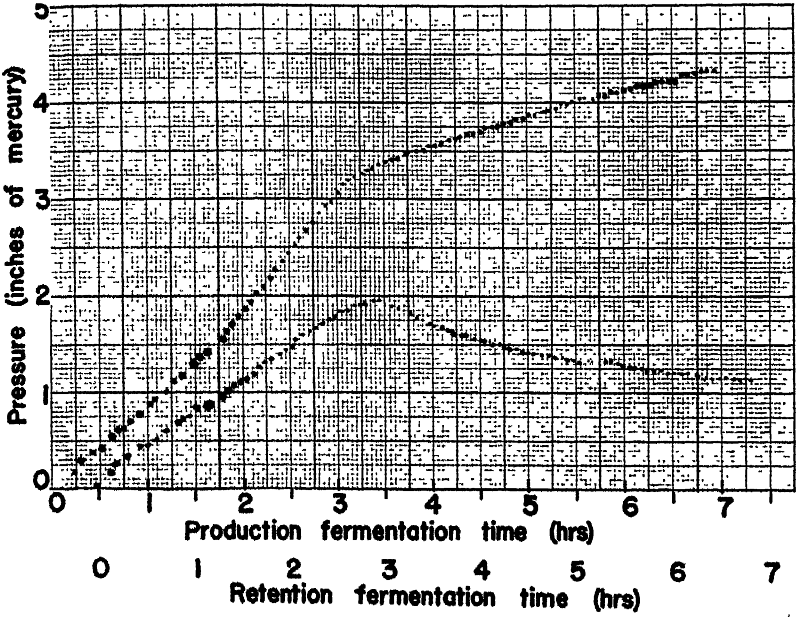


Fig. 2. Typical gas production (top) and gas retention curves obtained from automatic recording gasometer.

Both the production and retention curves are started at the same time; but because of the arrangement of the stylus, the production curve lags behind the retention curve. Correct comparison of the two curves requires adjustments for the starting points, with concomitant changes in the relative positions of the remainder of the curves.

The change in slope of the production curves is the point at which the reserve supply of fermentable sugars reaches a critically low level in so far as the rate of fermentation is concerned. The peak of the retention curve is at the point of maximum gas retention. Although the height of the curves at any point on the chart is directly measurable in inches, a simple calculation (15.88 × height in inches) transforms the height to milliliters of gas.

Experimental

The gassing power of winter, spring, and soft wheat flours was determined by both the Sandstedt and Blish (1934) manometric apparatus and the automatic recording apparatus described above on six consecutive days. The procedure described by Sandstedt and Blish (1934) was followed for the manometric method. The method is described in *Cereal Laboratory Methods* (4th ed., 1941). Only flour, water, and yeast were used. Two and one-half percent yeast and optimum absorption and mixing time were used for the automatic method. A 10-g aliquot of the dough, which was mixed by a regular mixograph apparatus, was used with the automatic apparatus.

In the comparative study of the manometric and automatic apparatuses only gas production comparisons were made. Gas retention measurements are possible and practical on the automatic apparatus, and frequently for gas retention studies regular bread formulae are used.

Readings were taken from the gauges of the manometric apparatus every half hour for a period of 6 hours (12 readings). As an aid in obtaining the pressure values from the automatic recording data, a flat glass on which parallel, vertical lines had been ruled was used to obtain the values recorded on the graph paper of the automatic recording gasometer. The lines were spaced at intervals equal to the distance the graph paper traveled on the rotating drum in half an hour. By placing the glass plate over the graph paper, with the first vertical line at the point of zero fermentation time, the succeeding intersections of the vertical lines on the glass plate with the graph recorded on the graph paper gave readings from the automatic recorder which were comparable with the readings from the manometric apparatus. It is these readings which have been used in comparing the two methods.

Results and Discussion

Table 1 gives the average values of the six replications, according to the type of flour, apparatus, and fermentation time. The manometric readings are in terms of gauge pressure. The automatic readings are in terms of units of mercury. Inasmuch as the graph paper used on the automatic apparatus was ruled 20 lines to the inch a unit of mercury pressure has been taken as 0.05 inch of mercury.

The linear relationship between the manometric and automatic readings was $Y = 4.58x$, where Y = manometric readings and x = automatic readings. The correlation between the data was + .978 which was of very high degree as well as highly significant. Only 4.4% of the variation was found to be independent of the correlation. The

TABLE I

MEAN VALUES OF READINGS FROM THE MANOMETRIC AND AUTOMATIC GASOMETERS

Reading No.	Fermentation time	Flour types						Experimental average	
		Soft		Spring		Winter			
		Method							
		Manometric apparatus[1]	Automatic apparatus[2]	Manometric apparatus	Automatic apparatus	Manometric apparatus	Automatic apparatus	Manometric apparatus	Automatic apparatus
	hrs.	*mm Hg*		*mm Hg*		*mm Hg*		*mm Hg*	
1	0.5	52	12	51	11	42	10	49	11
2	1.0	104	23	103	21	93	21	100	22
3	1.5	156	33	158	34	150	31	155	33
4	2.0	206	44	235	48	218	45	230	46
5	2.5	241	53	298	64	286	59	275	59
6	3.0	257	58	367	78	348	74	324	70
7	3.5	272	61	426	91	400	87	366	80
8	4.0	282	63	464	103	432	96	393	87
9	4.5	291	66	495	109	459	102	415	92
10	5.0	301	68	517	113	478	105	432	95
11	5.5	313	71	538	116	491	108	447	98
12	6.0	319	73	555	119	509	112	461	101

[1] Gauge pressure.
[2] Units of mercury (20 units = 1 inch mercury).
One unit of mercury = 1.29 mm.

correlations between the two methods, according to flour type, were + .959, + .983, + .983 for spring, winter, and soft wheat flour, respectively. The change in rate of gas production occurred at the same time with both methods.

The standard error, stated in terms of percentage of the respective means, of the manometric apparatus was 10.9% and that for the automatic apparatus was 6.8%.

The data thus indicate that the same results would be obtained with the automatic apparatus as with the regular A.A.C.C. method originally described by Sandstedt and Blish (1934). The automatic apparatus has the added advantages that it does not require the attention of an operator from the time it is started until it automatically shuts itself off and a new sample can be tested, and data are obtained with appreciably greater accuracy than with the regular method.

Summary

A new type automatic recording gasometer has been described. It was compared with the regular A.A.C.C. manometric method of determining gassing power.

The correlation between the two methods was of very high *degree* as well as highly significant (.978).

The standard errors were 10.9% and 6.8% for the regular manometric and automatic methods, respectively.

Literature Cited

Bailey, C. H., and Johnson, A. H.

1924 Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. Cereal Chem. 1: 293-301.

Blish, M. J., Sandstedt, R. M., and Astleford, G. R.

1932 Sugars, diastatic activity, and "gassing power" in flour. Cereal Chem. 9: 378-393.

Elion, E.

1939 Some remarks on the varying influence of compressed yeasts of different industrial origin on the gas retention of dough, as recorded by a new instrument, the Chelaro balance. Cereal Chem. 16: 598-610.

1940 The importance of gas-production and gas-retention measurements during the fermentation of dough. Cereal Chem. 17: 573-581.

Eva, W. J., Geddes, W. F., and Frisell, B.

1937 A comparative study of various methods of measuring flour gassing power. Cereal Chem. 14: 458 480.

Glabe, E. F.

1942 The fermentometer, a gas-production measuring device. Cereal Chem. 19: 230-234.

Heald, W. L.

1932 Some factors which affect gas production during dough fermentation. Cereal Chem. 9: 603-616.

Hullett, E. W.

1941 Convenient measurement of gassing power with dough under constant pressure and with elimination of gas solution. Cereal Chem. 18: 549-554.

Jørgensen, H.

1931 On the separation of "gassing power" (diastatic activity) from "strength" in baking tests. Cereal Chem. 8: 361-374.

Malloch, J. G.

1939 A convenient apparatus for gas production determinations by the Blish method. Cereal Chem. 16: 178-182.

Markley, M. C., and Bailey, C. H.

1932 An automatic method for measuring gas production and expansion in doughs. Cereal Chem. 9: 591-594.

Miller, H., Edgar, J., and Whiteside, A. G. O.

1943 An automatic gas recording apparatus. Cereal Chem. 20: 355-361.

Sandstedt, R. M., and Blish, M. J.

1934 Yeast variability and its control in flour gassing power tests. Cereal Chem. 11: 368-383.

Sherwood, R. C., Hildebrand, F. C., and McClellan, B. A.

1940 Modification of the Bailey-Johnson method for measurement of gas production in fermenting dough. Cereal Chem. 17: 621 626.

EFFECT OF FLOUR LIPIDS ON RECOVERY OF GLUTEN FROM HARD AND SOFT WHEAT FLOURS BY THE USE OF SULFITE SOLUTIONS[1]

IRWIN W. TUCKER

Enzyme Research Laboratory, Bureau of Agricultural and Industrial Chemistry,
Agricultural Research Administration, U. S. Department of Agriculture.

(Received for publication July 30, 1945)

The existence of a connection between flour lipids and some properties of flour is generally admitted, but the connection itself remains obscure. Most experimenters seem agreed that the extraction of lipids improves the properties of the bread; but there is less agreement on the effect produced by reincorporation of the extracted material.

Working (1924) increased the tenacity of poor gluten by washing phosphatides therefrom, and observed that the addition of phosphatide to normal flour injured the gluten quality. Similarly, Johnson and Whitcomb (1931) found that doughs (and breads) made from ether-extracted flour were superior to those from the originals; but the addition of lard to doughs made from the extracted flours markedly reduced their retention of gas. On the other hand, Sullivan, Near, and Foley (1936), while observing that the extraction of flour with ether did not reduce the loaf-volume or the quality of the dough, found that readdition of wheat lipids or fractions thereof also had no great effect on these properties (unless the lipids had become oxidized). Fisher and Jones (1936) found an improvement in flour owing to the removal of lipids, and a still further improvement when they were put back. Sinclair and McCalla (1937) have pointed out the importance of lipids to the characteristics of flours, and the experiments which they report show the complexity of the phenomena involved.

Changes in the lipid content of a flour are obviously reflected in the composition of the isolated crude gluten, because of the large quantities of lipids which the latter carries. Gluten prepared by Fisher and Halton (1933) contained about three-fourths of the total flour lipids.[2] Furthermore, Sullivan and Near (1927) observed the lipid content to be nearly the same in widely different wheats, and called attention to the fact that therefore the ratio of lipid to protein is much greater in soft than in hard wheat. Moreover, Baker and Mize (1942) found that the effect of added lipids on the baking qualities of natural flours depends more on the physical properties than on the chemical composition of the fatty material. While solid fats in general improved the texture and crumb more than did liquid fats, the composition of the solid was of minor importance, for certain waxes and hydrogenated true fats gave equally good results. It appears then that the effect of adding lipids to flour depends to a small extent upon the fatty material added, but to a large extent upon the type of flour used, i.e., hard or soft wheat.

---

[1] Enzyme Research Laboratory Contribution No. 98. Part of this work was done under Special Research Fund authorized by the Bankhead-Jones Act of June 29, 1935.

[2] Samples of sulfite gluten prepared in this laboratory from hard wheat flour and analyzed by Dr. W. G. Rose contained 6 to 9% of lipids, thus accounting for roughly two-thirds of the total in the flour. The glutens were hydrolyzed by acid or by papain before exhaustive extraction, and the extract was subsequently purified.

Experimental

A curious difference observed in this laboratory between hard and soft wheats is in the proportion of the total protein that can be recovered as a curd after agitating the flour with air and warm dilute sulfite solution according to the method of Balls and Tucker (1943). From hard wheat flours much of the protein can be recovered in this manner and washed until practically free from starch. With flour from soft wheats, however, similar treatment has resulted in low recoveries of protein, partly because less curd was formed and also because the curd did not cohere well enough to be washed thoroughly. It was therefore of interest to learn if a reduction in the lipid content of flour would lead to a better coagulation of the protein upon treatment with sulfite. This was found to be the case with soft wheat flour, but not with flour from hard wheat. The baking properties of the flour used are outside the scope of these experiments. There seems to be no obvious connection between the yield of protein recovered in this manner and the behavior of the flour in baking, other than the difference always implied between hard wheat and soft wheat flours.

In the experiments reported here, three unbleached patent flours were used; one was from hard and two from soft wheat. The hard wheat flour (A) was made from a winter wheat and contained 15.0% protein. The soft wheat flours were (B) a cracker flour, containing 10.4% protein, made from a western white wheat, and (C) a cookie flour, containing 6.5% protein, also made from a western white wheat.

Large quantities of each flour were extracted with low-boiling petroleum ether in glass percolators until the effluents were completely colorless.[3] As much solvent as possible was then removed by suction and the remainder was allowed to evaporate in a current of air without heating. The extracted lipids were concentrated by removing a large portion of the solvent on a steam bath. When it was desired to reincorporate some of the extracted lipids into the flour, the latter was mixed with a solution of the lipids in petroleum ether, and the solvent was allowed to evaporate spontaneously while the mixture was stirred in a current of air. In this way any desired proportion of the extract could be added, with good distribution, to the extracted flour.

---

[3] The extraction was assumed not to have removed the bound lipids; this is not strictly true, because substances resembling protamines have been found in the petroleum ether extract. As far as is known, however, their amount is relatively small.

The amount of extractable lipids was determined analytically in a Soxhlet apparatus, ethyl ether being used instead of petroleum ether, since the two solvents remove practically the same fat fraction. The lipid material remaining in the large batches of flour after extraction by petroleum ether was determined analytically by washing out the lipids with ethyl ether after the lipid-protein complexes had been broken down by long exposure to a mixture of ether and absolute alcohol. In detail, 100 g of previously extracted flour was shaken frequently for 48 hours at room temperature with 400 ml of a mixture of equal volumes of absolute alcohol and ether. The mixed solvents were filtered off, and the flour washed with ether. The combined liquids were evaporated directly to dryness, and the residue weighed. The results are given in Table I. The reason for accepting values obtained in this manner was that two other schemes of analysis by which the protein-lipid complexes were more obviously decomposed gave substantially the same results.[4] It is suggested that no present method for the determination of "bound lipids" in gluten is entirely beyond criticism; however, there is evidence that in the results presented here the easily extractable lipids play the larger role.

TABLE I

LIPID MATERIAL EXTRACTED FROM THREE FLOURS BY ETHYL ETHER AND THEREAFTER BY ETHER AND ALCOHOL

Flour	Protein in flour	Lipids extracted by ethyl ether	Lipids subsequently extracted by alcohol and ether	Total lipid
	%	%	%	%
A	15.0	0.99	0.31 [1]	1.30
B	10.4	1.00	0.30 [1]	1.30
C	6.5	1.10	0.33 [1]	1.43

[1] In the case of flour A, this fraction contained 0.92% of phosphorus (equivalent to 23% lecithin) and 0.92% of sterols. In the case of flour B, it contained 0.73% of phosphorus (equivalent to 18% lecithin) and 1.2% of sterols. Flour C contained 0.97% of sterol. Phosphorus was determined by the method of Fiske and Subbarow (1925); sterols by the method of Pijoan and Walter (1937).

The protein was coagulated by putting 8 liters of water containing 8 g of a mixture of sodium sulfite and sodium acid sulfite (in the proportion to give a pH of 6.0, when dissolved) into a 15-gallon barrel churn, and then adding 2400 g of the flour. The churn was rotated for 30 minutes at 45 rpm The temperature of the contents fell from 40° to 35°C. After churning, the coagulated protein was permitted to rise to the top, washed thoroughly with cold water, and then denatured by heating to 100° while suspended in about 5 liters of water. The denatured protein was filtered off on cheesecloth, dried, and analyzed for nitrogen.

---

[4] A 12.5-g portion of flour A was digested with 0.13 g of a very active papain paste in 50 ml of water containing 1 ml of 2 *M* KCN for 18 hours at room temperature. The digest was then diluted with 300 ml of a 1:1 mixture of normal alcoholic HCl and ether. After 5 hours the mixture was poured into 1.5 liters of water. The ether layer was removed. The aqueous layer was extracted twice more with suitable quantities of ether, and the combined ether extracts were then washed thrice with water. The ether was dried over sodium sulfate and evaporated. The residue was weighed. By this method 0.29% of the previously extracted flour was recovered as lipids.

In another experiment a 25-g portion of flour A was treated for 48 hours at room temperature with a mixture of 150 ml *N* HCl in absolute alcohol and 150 ml ether. The flour was filtered off and washed well with ether. The combined liquids were evaporated to about 25 ml and then poured into about 500 ml of water. The watery emulsion was repeatedly extracted with ether; the ether was dried and evaporated. The residue, amounting to 0.35% of the flour, was recovered. From flour B, 0.32% was obtained by the same procedure.

TABLE II

PROTEIN RECOVERED FROM FLOURS BEFORE AND AFTER EXTRACTION WITH PETROLEUM ETHER

Material	Protein present	Recovery of protein present in	
		Natural flour	Extracted flour
	%	%	%
Flour A	15.0	65	53
Flour B	10.4	0	44
Flour A, 67% + 33% starch	10 4	17	41
Flour B, extracted also with alcohol-ether	10.4	-	43
Flour B, 1.4 times the usual concentration in suspension[1]	10.4	20	—
Flour C	6.5	0	44
Flour A, 43% + 57% starch	6.5	0	30
Whole (hard) wheat meal	16.2	37.5	42
Feed middlings	12.6	0	10

[1] 2800 g flour in 6.6 liters of sulfite solution.

Table II shows the percentage of the total flour protein that was recovered by this means. Because of the difficulty in handling small batches of gluten, especially when the clot is granular (as is the case sometimes with sulfite solutions), no great degree of accuracy can be claimed for the yield values. Repeated experiments not reported here indicated that these values are only reproducible to about ±5% of the flour used; that is to say, two independent measurements will vary at most by about 10%, without the variation being considered significant here. However, the flour lipids are in some way connected with such spectacular changes in the yield of protein that a greater accuracy of measurements is hardly needed to demonstrate them, even if it were practicable.

Discussion

It may be seen that the fraction of protein recovered was increased from nothing to about 40% by removing the extractable lipids from the soft wheat flours. But the yield of protein recovered never rose to the level of that from the hard wheat flour under the conditions of these experiments. The amount of protein recovered from hard wheat was not greatly affected by removal of the extractable lipids.

When the lipids removed by petroleum ether (Table III) were reincorporated with extracted soft wheat flour, the yield of protein recovered was greatly lessened. As little as one-quarter of the lipid material originally extracted reduced the quantity of recoverable protein to one-third of its former value. In the case of the hard wheat flour, however, the addition of 1.5 times the quantity of lipid material originally extracted was required to reduce the yield of protein to two-thirds.

TABLE III

EFFECT OF ADDED LIPIDS ON YIELD OF PROTEIN FROM FLOUR EXTRACTED WITH PETROLEUM ETHER

Material	Protein present	Total lipid	Protein recovered	
			Added lipid from same flour	Added lipid from the other flour
	%	%	%	%
Flour A, no lipid added	15.0	0.3	53	
Flour A, flour lipid added	15.0	0.8	61	
Flour A, flour lipid added	15.0	1.3	58	56
Flour A, flour lipid added	15.0	1.8	11	
Flour A, flour lipid added	15.0	2.3	0	8
Flour A, cottonseed oil added	15.0	2.3	0	
Flour A, water extract from an equal weight of flour B added	15.0	1.3	15	–
Flour B, no lipid added	10.4	0.3	44	
Flour B, flour lipid added	10.1	0.6	16	32
Flour B, flour lipid added	10.1	0.8	0	
Flour B, flour lipid added	10.4	1.3	0	0
Flour B, flour lipid added	10.4	1.8	0	-

The foregoing experiments show that the proportionate yield of coagulable protein from a soft wheat flour could be increased by removing part of the lipids. It could also be enlarged by increasing the concentration of protein present without changing the lipid-protein ratio. This was done by reducing the amount of liquid used with a low protein flour (B) so that the concentration of protein was the same as that for the high protein flour in the larger volume. By this means the yield of protein from flour B was increased (Table II), but not enough to equal the yield from flour A. Furthermore, mixtures of wheat starch and high protein flour (A) were made so that the protein of the mixtures corresponded to that found in flours B and C, respectively. These mixtures behaved very like the natural flours of the same (low) protein content (Table II).

There is thus a great difference in the extent to which small quantities of easily extracted flour lipids affect the yield of gluten from hard and soft wheat flour.[5] A difference in the composition of the fatty material extracted from soft wheat flour is not responsible for the low yield of protein therefrom, because this yield is equally depressed by the material extracted from hard wheat flour. A similar method of procedure was used by Finney (1943) to show that the difference in baking quality between Kharkof and Chiefkan wheat was not due to the specific nature of the extractable lipids. In considering the yield of coagulated gluten, it seems clear that the amount of lipid present in the flour is a significant factor, but the critical amount varies with the type of flour.

On the other hand, there is no evidence that the proteins in the two types of flour are responsible for their different behaviors toward sulfite solution. The addition of the water-soluble proteins from soft wheat flours had little influence on the amount of protein recovered from hard wheat flour (Table III), showing that this fraction of the soft wheat did not carry with it an anticoagulating factor sufficient to account for the differences observed between the two flours. This viewpoint is supported by the observation (Table II) that when the concentration of protein in high-protein flour was reduced by the addition of wheat starch, the mixture behaved like a flour of naturally low-protein content. But the ratio of fat to protein in the case of these artificial mixtures is not the same as for low-protein flour; on the contrary, it is the same as in the original high-protein flour. This indicates that the lipid-to-protein ratio does not alone determine the yield of protein. Flour A tolerated about twice as much lipid to protein as flour B before the yield of protein vanished.

Because of the ease with which some of the lipid material may be removed by solvent from natural flour, it seems reasonable that such lipids exist either free or on the surfaces of protein particles. In each case an accumulation of lipids at the surface of the protein can be expected when the flour is mixed with water, because so much of the fatty material, whether naturally present or artificially added, ultimately appears in the gluten. It is probable, therefore, that the proportion of easily extractable lipid to protein indicates the superficial concentration of lipids on the protein particles while the latter are still small. The higher this superficial concentration of lipids, the lower will be the number of collisions that "stick" during the churning. On the other hand, the total number of collisions between protein particles must depend on their concentration, and that is roughly represented at the start by the protein content of the flour. An increase in the protein content of the flour should therefore increase the number of collisions, while an increase in the proportion of extractable lipid to protein should decrease their individual effectiveness. If, as seems likely, the surface of the protein particles is the critical area involved, soft wheat flour may be expected to give lower yields of protein.

---

[5] If the lipid material remaining in the flour after simple extraction were responsible for the observed differences, it is difficult to understand why the yields of protein from extracted soft wheat flour were so high, and so readily lowered by returning a trace of the extracted material to the flour. Furthermore, as shown in Table II, the soft wheat flour gave the same yield of coagulated protein after extraction with both petroleum ether and alcohol-ether as it gave after extraction with petroleum ether alone, showing that the residual lipoproteins played only a minor role in the coagulation of the protein.

Several factors not presently determinable would have to be known before drawing any quantitative picture from these or similar data. The sizes of the protein particles during the essential steps of the coagulation would have to be known, and also the fate of the surface lipid during that period, how much is buried in the coagulum, how much is concentrated on the new (and smaller) surface, and how much is given up to the surrounding medium. Furthermore, while the present data are too approximate to indicate it, some involvement of the chemical properties of both the lipids and proteins present is of course to be anticipated. On the other hand, if this hypothesis is assumed in principle, it must be admitted that the hard wheat flour has behaved in these experiments as though its protein particles were on the average decidedly smaller during the coagulation process than those of the soft wheat flour.

Summary

Three patent flours, one from hard wheat and two from soft wheats, were extracted with petroleum ether. The removal of lipids from soft wheat flour greatly increased the amount of coagulable protein that could be recovered on treatment with sulfite solution. The removal of lipids from hard wheat flour did not alter the yield of protein therefrom.

The addition of the flour lipids to previously extracted flour resulted in a smaller yield of protein, regardless of the type of flour from which the lipids were prepared. The amount of artificially added lipid necessary to reduce the yield of protein materially was very small in the case of the soft wheat flour; two or three times as much was required to produce a commensurate decrease in the yield of protein from hard wheat flour.

An "anticoagulating" action sufficient to explain the results could not be demonstrated in either the lipid or the protein fractions of the soft wheat flour.

The results suggest an explanation based on the hypothesis that the coherence of protein particles to form a coagulum (and possibly also a dough) varies directly with the number of the protein particles and inversely with the surface concentration of lipids thereon.

Literature Cited

Baker, J. C., and Mize, M. D.
1942 The relation of fats to texture, crumb, and volume of bread. Cereal Chem. **19**: 84–94.
Balls, A. K., and Tucker, I. W.
1943 The extraction of diastase and recovery of protein from wheat. Fruit Products J. and Am. Food Manufacturer **23**: 15 16, 21.
Finney, K. F.
1943 Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. **20**: 381–396.
Fisher, E. A., and Halton, P.
1933 Effect of the addition of oils and fats to doughs on the composition of the washed-out glutens. J. Soc. Chem. Ind. Trans. **52**: 195–198.
—— and Jones, C. R.
1936 Note. Cereal Chem. **13**: 496.
Fiske, C. H., and Subbarow, Y.
1925 The colorimetric determination of phosphorus. J. Biol. Chem. **66**: 375–400.
Johnson, A. H., and Whitcomb, W. O.
1931 Wheat and flour studies. XIX. Studies of the effect on their bread-making properties of extracting flours with ether, with special reference to the gas retaining powers of doughs prepared from ether-extracted flours. Cereal Chem. **8**: 392 403.
Pijoan, M., and Walter, C. W.
1937 Determination of cholesterol. J. Lab. Clin. Med. **22**: 968.
Sinclair, A. T., and McCalla, A. G.
1937 The influence of lipoids on the quality and keeping properties of flour. Can. J. Research **C15**: 187–203.
Sullivan, B., and Near, C.
1927 Relation of the magnesium in the ash and the lipoid-protein ratio to the quality of wheats. J. Am. Chem. Soc. **49**: 467–472.
——, ——, and Foley, G. H.
1936 The role of the lipids in relation to flour quality. Cereal Chem. **13**: 318–331.
Working, E. B.
1924 Lipoids, a factor influencing gluten quality. Cereal Chem. **1**: 153–158.

CEREAL CHEMISTRY

VOL. XXIII MAY, 1946 No. 3

GRAIN STORAGE STUDIES. III. THE RELATION BETWEEN MOISTURE CONTENT, MOLD GROWTH, AND RESPIRATION OF SOYBEANS[1]

MAX MILNER[2] and W. F. GEDDES[3]

[illegible]tural Biochemistry, Minnesota Agricultural Experiment
[illegible], University Farm, St. Paul, Minnesota

([illegible]eived for publication January 30, 1946)

Moisture content is recognized as the most critical of all factors influencing the respiration, heating, and deterioration of stored grain. As the moisture content of dry grain is increased, there is a relatively slight increase in the respiratory rate until a critical moisture range, characteristic of the seed species and influenced by factors related to the commercial quality of the grain, is reached above which a rapid acceleration in respiration occurs. This has been demonstrated by Kolkwitz (1901) with barley, by Bailey and Gurjar (1918) with wheat, by Bailey (1921) with corn, by Bailey (1940) with flaxseed, rye, and other cereal grains, and by other workers with other seed types. At moisture contents favorable to rapid respiration, the heat production may be sufficient to increase the temperature of grain stored in bulk and thereby accelerate its deterioration.

Bailey and Gurjar (1918) explained the marked increase in respiration above the critical moisture range by assuming the formation of a continuous gel in the kernel structures which facilitates diffusion of the soluble nutrients to the respiratory centers of the seed located principally in the seed embryo. Bailey (1940) and Kretovitch and Uschakova (1940) admit that molds may contribute to grain respiration but only when the moisture content is well above that at which the initial rapid increase in grain respiration occurs. On the other hand, many workers including Cohn (1890), Darsie, Elliot, and Peirce (1914), McHargue (1920), Thom and LeFevre (1921), Gilman and Barron (1930), Swanson (1934), Larmour, Clayton, and Wrenshall (1935), Koehler (1938), Ramstad and Geddes (1942), Milner, Warshowsky, Tervet, and Geddes (1943), Leach (1944), and Milner and Geddes (1945) have stressed the importance of saprophytic mold microflora which are almost invariably present on grain, in contributing to the respiration and associated deteriorative changes. That the so-called "critical moisture content" of seeds may be associated with minimum moisture levels at which certain common molds will germinate and grow has been suggested by Gilman and Barron (1930). The work of Thomas (1937) with wheat and of Tervet (1945) with soybeans indicates that saprophytic fungi, particularly certain members of the *Aspergillus* species, may also be responsible for losses in the viability of stored grain.

---

[1] Paper No. 2274, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, March, 1945.

[2] Research Associate, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

[3] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

The present study was undertaken to investigate the interrelationships between moisture content, mold growth, seed viability, respiration, and changes in the chemical composition of soybeans. The respiratory characteristics of soybeans of varying grade were determined at various moisture levels by a method which provided for the constant aeration of the samples at constant temperature. At the end of the respiration trials which extended over time periods up to 15 days, the seeds were examined to determine the extent of mold growth and chemical deterioration. A study also was made of the respiration of autoclave-sterilized soybeans inoculated with *Aspergillus flavus*, a fungus commonly present in commercial samples

Materials and Methods

The soybeans used in this study included: (1) a seri moist soybeans of the Illini variety grown in Illinois in 1945, (2) an exceptionally sound, well-matured sample of Wisconsin Manchu soybeans grown for seed purposes at University Farm, St. Paul, Minnesota, in 1943; and (3) a very severely frost-damaged immature sample of soybeans (sample grade, damage 55%, germination 5%) which was a composite representing the poorest quality soybeans grown in Minnesota in 1942.

Respiratory rates and respiratory quotients exhibited by the various soybean samples were determined using the apparatus and air analysis technique described by Milner and Geddes (1945), whereby the seed samples, maintained in respirometer bottles in a thermostat, were aspirated continuously with air at a relative humidity which was in hygroscopic equilibrium with the initial moisture content of the samples. Analysis of the effluent air for oxygen and carbon dioxide was performed daily.

Except in the case of the naturally moist series of soybeans, the seed samples were conditioned to the required moisture levels by the direct addition of water, as described in a previous communication (Milner and Geddes, 1945). Moisture values were determined by the two-stage air-oven method outlined in Service and Regulatory Announcements No. 147, issued by the Agricultural Marketing Service, U.S.D.A.

Estimation of the number of seeds in a given sample infected with molds, as well as identification of the prevalent mold types, was carried out by the surface-sterilization and plating technique described by Milner, Warshowsky, Tervet, and Geddes (1943).

Oil acidity, expressed as acid value (milligrams of potassium hydroxide required to neutralize one gram of oil), was determined on the oils extracted from soybeans by the method of Zeleny and Coleman (1938). Analyses for total, reducing, and nonreducing sugars in various soybean samples subjected to respiration trials were carried out on finely ground seed samples by the methods outlined for wheat flour in *Cereal Laboratory Methods* (4th ed., 1941).

Germination values reported for certain of the soybean samples were furnished by the State Seed Testing Laboratory, University Farm, St. Paul 8, Minnesota.

Respiration of Naturally Moist Soybeans

The respiratory rates of six samples of Illini soybeans containing from 8.5% to 14.6% moisture were determined at 37.8°C over an 11-day period with samples weighing 250 g. To avoid the possibility of inhibitory carbon dioxide concentrations in the interseed atmosphere and at the same time to obtain readily measurable changes in the composition of the effluent air, two aeration rates were employed, namely, 1000 ml per day for samples containing 14.0% moisture and more, and 500 ml per day for the samples of lower moisture content.

The carbon dioxide values are given in Figure 1 in the form of the respiration-time curves for the various moisture levels. The relation between respiratory rate and moisture content on the eleventh day is also shown.

That inhibition of respiration was not involved in this trial is indicated by the data of Table I. The maximum carbon dioxide concentration in the interseed air was 1.02% which is well below the initial inhibitory level of approximately 12% previously reported by Milner and Geddes (1945).

The importance of continuous adequate aeration and of prolonging seed respiration trials for several days, stressed by Milner and Geddes (1945), is confirmed by the results of this trial. The respiration of the soybean samples containing 14% moisture and less remained low and

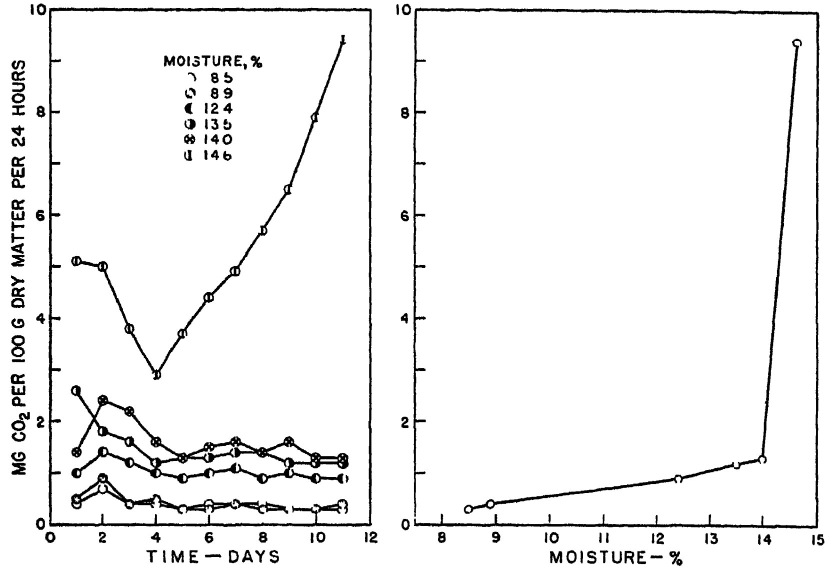


Fig. 1. Influence of time and moisture content on the respiratory rate of naturally moist Illini soybeans at 37.8°C.

relatively constant throughout the 11-day period. In contrast, after an initial decline in carbon dioxide output during the first four days, the respiratory rate of the sample at 14.6% moisture broke upwards and continued to accelerate throughout the remainder of the trial. The maximum respiratory rate of this sample was therefore not attained. The initial decline in respiratory rate shown by this sample over a period of several days may only be an apparent one since raising the temperature of the soybeans from room temperature to that of the thermostat (37.8°C) would result in the evolution of some of the pre-

TABLE I

EFFECT OF NATURAL MOISTURE CONTENT ON THE RESPIRATORY RATE OF ILLINI SOYBEANS

(Data are for the eleventh day of trial at 37.8°C with sample weights of 250 g)

Moisture	Aeration per day	CO in interseed air	Respiratory rate[1]
%	*ml*	%	
8.5	500	0.07	0.3
8.9	500	0.09	0.4
12 4	500	0.20	0.9
13.5	500	0.26	1.2
14.0	1000	0.14	1.3
14.6	1000	1.02	9.4

[1] Milligrams CO_2 per 100 g dry matter per 24 hours

viously respired carbon dioxide retained by the seed. A similar apparent decrease in respiration was noted by Milner and Geddes (1945) in respiration trials with soybean samples freshly conditioned to various moisture levels, the decrease in initial respiratory rate being more pronounced as the moisture content of the samples increased. A similar phenomenon with soaked pea seeds has been observed by Gane (1935) who suggests that it is "probably due to an accumulation of carbon dioxide during soaking of the seed preliminary to the respiration trials."

The marked acceleration in the respiratory activity of the seeds containing 14.6% moisture, after the fourth day, as contrasted to the very low and relatively constant respiratory rates of the samples at moisture contents between 8.5% and 14.0%, suggests that the activation of some additional biological factor occurs between the narrow moisture range of 14.0 and 14.6%. Examination of the seeds at the end of the trial disclosed that only the sample at 14.6% moisture showed visible mold growth. According to the data of Ramstad and Geddes (1942), a moisture content of 14% in soybeans is in equilibrium with an atmospheric relative humidity of 74.7%, while a moisture value of 14.6% is in equilibrium with a relative humidity of 76.2%. Several investigators including Lea (1933), Galloway (1935), Barton-Wright and Tomkins (1940), and Macara (1943) have shown that over relatively short time intervals, a relative humidity of 75% is a minimum for mold spore germination at ordinary temperatures in the presence of nutrients.

The inflection of the moisture-respiration curve is much sharper than that obtained with various grains by previous investigators who usually carried out their studies over time intervals of only a few days and either aspirated off the accumulated carbon dioxide at intervals, or at the end of the trials. The present data suggest that former methods tended to underestimate the maximum respiratory potential because of the short duration of the trials, combined, in some instances, with suppression of respiration by inhibitory carbon dioxide concentrations in the interseed air, particularly at the higher moisture levels.

Respiration of Artificially Dampened Soybeans

High-Quality Soybeans. Subsamples of a sound, well-matured lot of Wisconsin Manchu seed, showing 94% germination and very low microfloral infection, were conditioned to moistures ranging from 11.8 to 18.3% and respiration trials were carried out at 37.8°C with 250 g samples aerated at 2000 ml per day, in the presence of the appropriate air humidifying solutions. The moisture contents of the samples, assayed before and after the trial, showed a maximum variation of

0.2%. The respiration data which are presented in Figure 2 emphasize the importance of the time factor in any measurement of respiratory potential. The concentrations of the carbon dioxide in the interseed air and the respiratory quotients for the two highest moistures (16.0 and 18.3%) are given in Table II for the last four days of the trial.

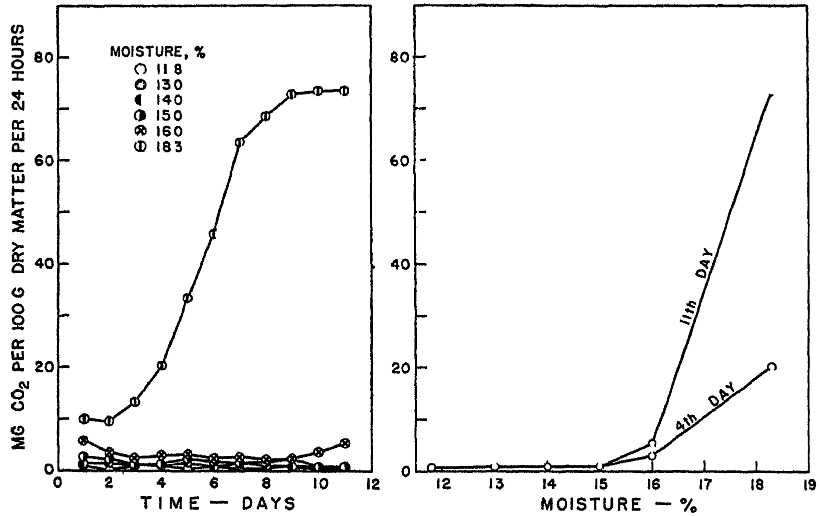


Fig 2 Influence of time and moisture content on the respiratory rate of artificially dampened Wisconsin Manchu soybeans at 37 8°C

TABLE II

RELATIONSHIP BETWEEN MOISTURE CONTENT, CARBON DIOXIDE CONCENTRATION IN THE INTERSEED AIR, AND RESPIRATORY QUOTIENT EXHIBITED BY HIGH-QUALITY WISCONSIN MANCHU SOYBEANS RESPIRING AT 37.8°C

Moisture		Day of trial			
%		8	9	10	11
16.0	CO_2 in interseed air, %	0.12	0 13	0 20	0.29
	R.Q.	0.75	0 72	0 87	0 78
18.3	CO_2 in interseed air, %	3 57	3 77	3 80	3.80
	R.Q	0.98	0.95	0 99	0.97

Respiratory quotient values for the samples at lower moistures are not considered reliable because of the extremely small percentages of gases exchanged at the rate of aeration applied.

Initial and final moisture values, oil acidity, germination data, and observations on the extent of mold growth are reported in Table III.

TABLE III

EFFECT OF MOISTURE CONTENT ON RESPIRATION, ACID VALUE OF OIL, VIABILITY, AND MOLD GROWTH

(High-quality Wisconsin Manchu soybeans after eleven days respiration at 37.8°C)

Moisture		Respiratory rate [1]	Acid value of oil [2]	Germination	Visual condition
Initial	Final				
%	%			%	
11.8	(original sample)	—	1.0	94	Mold-free
11.8	11.9	0.5	1.0	85	Mold-free
13.0	13.2	0.9	1.1	74	Mold-free
13.9	14.0	0.9	1.3	60	Mold-free
15.1	15.0	0.9	1.5	43	Mold-free
16.0	16.0	5.4	1.8	15	Mold-free
18.3	18.3	73.3	6.9	0	Moldy

[1] Milligrams CO_2 per 100 g dry matter per 24 hours on eleventh day of trial.

[2] Milligrams of potassium hydroxide required to neutralize the acidity of 1 g of oil.

All samples at moisture values below 16.0% showed very low and relatively constant respiratory rates throughout the trial. That at 16.0% moisture gave slight respiratory increases commencing on the ninth day, whereas the one at 18.3% moisture yielded rapidly increasing respiratory activity after the second day, reaching approximate equilibrium on the ninth day. The critical moisture for this series appeared to lie between 15.0 and 16.0% (77.3% to 79.5% relative humidity) as compared to 14% (74.8% relative humidity) shown by the naturally moist series.

Visual examination of the seeds after the trial showed only the highest moisture sample (18.3%) to be moldy, although the slight increase in respiration of the sample at 16.0% moisture during the final few days of the trial indicated that mold growth was beginning. In this connection Larmour, Sallans, and Craig (1944) have pointed out that the marked increases in respiration as a result of mold growth appear well before the mold is visible to the naked eye.

Table II suggests that the respiratory quotient of soybean respiration at moisture levels favorable to mold growth increases with moisture content.

Marked increases in oil acid value of respiring soybeans did not occur until the moisture content exceeded that at which mold growth begins (Table III). Loss of viability, however, occurred throughout the entire moisture range, but was accentuated at moisture values conducive to mold growth.

Severely Damaged, Low-Quality Soybeans. Subsamples of the frost-damaged lot of soybeans were conditioned to five moisture values in the range 10.1% to 17.0%. In view of the high degree of microfloral contamination shown by this sample which would be expected to cause high respiration values (Milner, Warshowsky, Tervet, and Geddes, 1943), seed samples of only 150 g were used and the maximum aeration rate, 2000 ml per day, was applied.

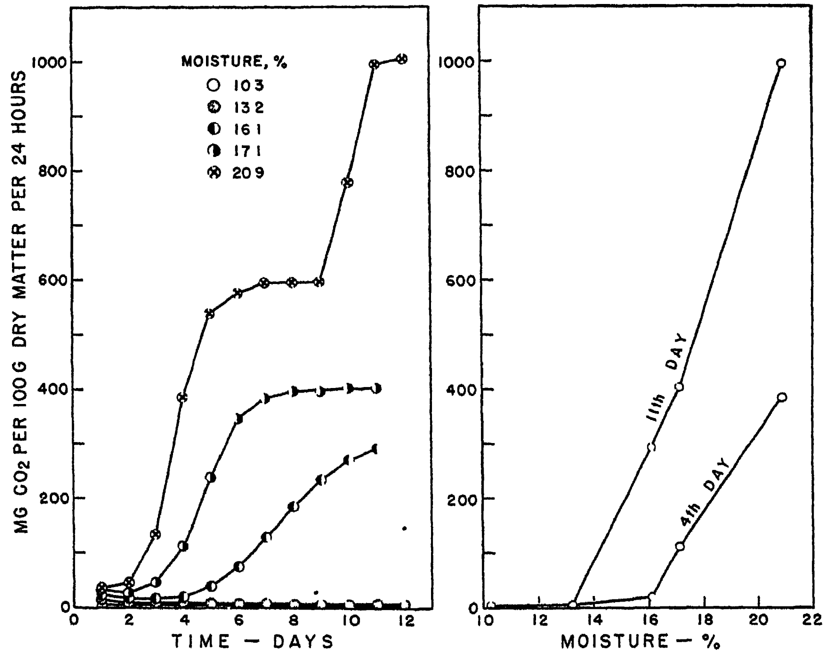


Fig. 3 Influence of time and moisture content on the respiratory rate of artificially dampened, severely frost damaged soybeans at 37.8°C.

The data for the trial given in Figure 3 indicate that in spite of the high ratio of aeration to sample size, the respiration of the highest moisture sample was so great that by the sixth day the concentration of carbon dioxide in the interseed air reached levels inhibitory to respiration. On the ninth day, half of this sample was removed, thus effectively doubling its aeration. The relation between moisture content, concentration of carbon dioxide in the interseed air, and respiratory quotient for the last six days of the trial for the three samples of the highest moisture content is given in Table IV. Chemical, moisture, and other changes in the seeds as a result of the respiration trial are shown in Table V.

The data indicate that extremely high respiratory rates can occur in highly damaged seeds at moisture values favorable to mold growth. In this case the critical moisture value lay between 13.2% and 16.0%,

TABLE IV

RELATIONSHIP BETWEEN MOISTURE CONTENT, CARBON DIOXIDE CONCENTRATION IN THE INTERSEED AIR, AND RESPIRATORY QUOTIENT EXHIBITED BY SEVERELY FROST-DAMAGED SOYBEANS RESPIRING AT 37.8°C

Final moisture		Day of trial					
		6	7	8	9	10	11
%							
16.1	CO_2 in interseed air, %	2.38	4.15	5.98	7.52	8.68	9.36
	R.Q.	0.82	0.82	0.87	0.89	0.89	0.90
17.1	CO_2 in interseed air, %	10.94	12.15	12.65	12.61	12.73	12.71
	R.Q.	0.90	0.90	0.91	0.88	0.91	0.91
20.9	CO_2 in interseed air, %	17.39	17.90	17.97	18.05	11.80	15.03
	R.Q.	0.93	0.94	0.94	0.94	0.94	0.96

TABLE V

EFFECT OF MOISTURE CONTENT ON RESPIRATION, CHEMICAL COMPOSITION, VIABILITY, AND MOLD GROWTH

(Severely frost-damaged soybeans after 12 days respiration at 37.8°C)

Moisture		Respiratory rate [1]	Acid value of oil	Total sugars	Reducing sugars as % of total [2]	Germination	Visual condition
Initial	Final						
%	%			mg/10 g	%	%	
6.8	(original sample)	—	2.9	—	—	5	Mold-free
10.2	10.3	0.6	3.8	290	44.1	6	Mold-free
13.1	13.2	3.3	4.2	291	43.6	0	Mold-free
15.1	16.1	291.7	14.5	268	51.1	0	Some mold
16.1	17.1	400.9	22.7	239	57.3	0	Moldy
17.0	20.9	1003.8	56.4	219	70.3	0	Very moldy

[1] Milligrams CO_2 per 100 g dry matter per 24 hours on twelfth day of trial.

[2] Reducing sugars are calculated as maltose, nonreducing as sucrose.

apparently well below the 16% value for the high-quality seeds (Figure 2). These results appear to be in line with the findings of Tomkins (1929) since they demonstrate that increased nutrient availability to the molds, as might be expected with frost-damaged seeds, tends to lower the humidity requirements for mold spore germination. Such an increase in the readily available nutrients present on the damaged seeds would result also in a greater extent of mold proliferation, as evidenced by the very high respiratory rates reached by the damaged sample. Thus sound seeds such as those of the high-quality sample, with their smooth unbroken seed coats, present a more inhospitable medium for mold mycelial penetration and growth than do the highly frost-damaged seeds, in which the majority of the seed coats were ruptured, and a large part of the sample consisted of naked cotyledons entirely devoid of seed coats. At a moisture content of 18.3% the high-quality sample showed a maximum respiratory rate of 73.3 mg carbon dioxide per 100 g dry weight per 24 hours; while by interpolation, the frost-damaged sample has a rate of about 600 mg at this moisture level. These values indicate that a much greater hazard is involved in the storage of damaged as compared with sound damp soybeans.

The tendency of the respiratory quotient to increase with moisture contents corresponding to relative humidities favorable to mold growth, as noted in the previous experiment, is also shown by the data in Table IV. Milner and Geddes (1945) observed a similar trend with samples of approximately equal moisture content (18.5 to 19.3%) when the aeration rates were increased from low values at which respiratory inhibition occurred.

The very marked increases in the respiratory rate of damaged seeds with time, at moisture values permitting mold growth, are reflected in profound changes in chemical composition (Table V). The greatest relative change occurred in the degree of fat hydrolysis, as indicated by the acid values of the extracted oils. Total sugars decreased while reducing sugars underwent a marked increase.

A striking result of this experiment, in which respiratory rates reached very high rates, was the marked increase in the moisture content of samples on which mold growth occurred.

The Respiratory Behavior of Sterile Soybeans Inoculated with Aspergillus flavus

Mycological examination of the high-quality Wisconsin Manchu seeds at the end of the respiration trials showed that the sample at 18.3% moisture was contaminated with *Aspergillus flavus* and *A. glaucus*. *A. flavus* has been shown by Thomas (1937) and Tervet (1945) to exert a toxic effect on seed viability. These observations prompted a study of the respiratory characteristics of this mold by inoculating sterile soybeans with spores grown in pure culture. By this means any biological activity due to the seeds themselves was eliminated.

Six lots of the high-quality samples were conditioned to various moisture contents in stoppered Erlenmeyer flasks for four days at room temperature, with occasional shaking. With cotton plugs replacing the stoppers, the samples were autoclaved for one hour at 120°C. A slight browning of the seeds resulted from this treatment. In the same autoclave were placed a number of eight-ounce bottles to be used as respirometers, which were previously tared with the stoppers and connections used for assembling the respirometers. All openings to the respirometers were previously plugged with glass wool. Observing sterile precautions, the sterile cool seeds were inoculated with a large number of spores of *Aspergillus flavus* from a week-old pure culture grown on an agar slant. Five samples of varying moisture content were inoculated with mold spores, while a sixth, at the highest moisture, was left sterile to serve as a control during the respiration trials. Portions of the inoculated samples were transferred to the tared respirometer bottles which were closed with the sterile stoppers through which passed the glass wool plugged connections. Seed sample weights in the respirometer bottles were determined by weighing the sealed bottles and their contents. The samples ranged in weight from 109 to 146 g.

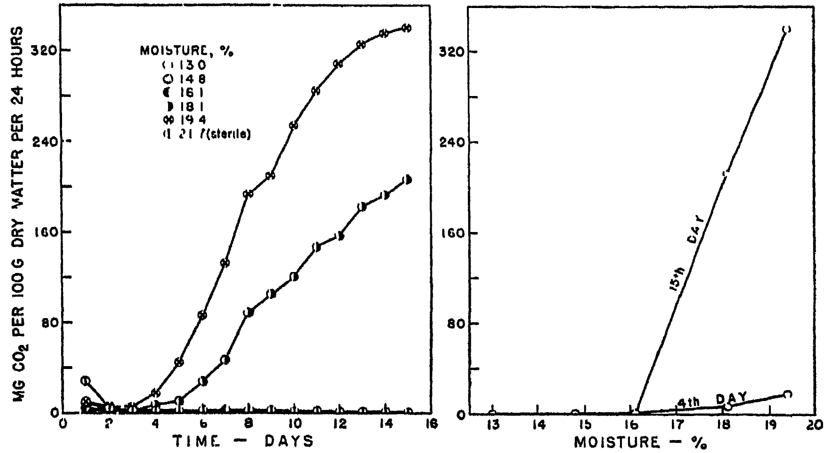


Fig 1. Influence of time and moisture content on the respiratory rate of sterile soybeans inoculated with *Aspergillus flavus* at 37.8°C.

Respiration trials were conducted at 37.8°C and an aeration rate of 2000 ml per day, with results shown in Figure 4. The relationship between moisture content, carbon dioxide concentration in the interseed atmosphere, and respiratory quotient for the inoculated samples at the three highest moisture levels during the last four days of the trial is shown in Table VI. Changes in the chemical composition and the visual condition of the seeds are recorded in Table VII. The initial chemical values are for determinations made on the samples after autoclaving but before the respiration trial; any effects of autoclaving on chemical composition are not involved.

The close similarity of the respiratory characteristics of a pure mold culture growing on a sterile soybean substrate to those of normal seeds at similar moisture values indicates that the greatest part of the

TABLE VI

Relationship Between Moisture Content, Carbon Dioxide Concentration in the Interseed Air, and Respiratory Quotient Exhibited by Autoclave-Sterilized Soybeans Inoculated with *Aspergillus flavus*, at 37.8°C

Final moisture		Day of trial			
		12	13	14	15
%					
16.1	CO_2 in interseed air, %	0.00	0.00	0.00	0.00
	R.Q.	—	—	—	—
18.1	CO_2 in interseed air, %	3.58	3.93	4.36	4.83
	R.Q.	0.98	0.97	1.01	1.00
19.4	CO_2 in interseed air, %	6.96	7.35	7.58	7.69
	R.Q.	0.99	0.98	0.99	0.99

TABLE VII

Effect of Moisture Content on Respiration, Acid Value of Oil, and Mold Growth

(Autoclave-sterilized Wisconsin Manchu soybeans inoculated with *Aspergillus flavus*, after 15 days respiration at 37.8°C)

Moisture		Respiratory rate[1]	Acid value of oil		Visual condition
Initial	Final		Initial	Final	
%	%				
12.9	13.0	0.0	1.3	1.0	Mold-free
14.8	14.8	0.0	1.3	1.0	Mold-free
16.4	16.1	0.0	1.3	1.0	Mold-free
17.4	18.1	212.0	1.1	1.9	Moldy
18.9	19.4	340.2	1.3	3.8	Moldy
21.9 (sterile control)	21.7	0.3	1.5	1.1	Mold-free

[1] Milligrams CO_2 per 100 g dry matter per 24 hours on fifteenth day of trial.

sharp increase in respiration of normal wetted seeds can be attributed to mold proliferation commencing at a sharply defined minimum humidity. The small but measurable amount of respiration noted with normal seeds at low moisture values is absent in the sterile mold-inoculated seeds in a similar moisture range and suggests that this very slight carbon dioxide evolution is due to seed respiration.

The critical point in the moisture-respiration curve occurred slightly above 15% for the normal, sound sample (Figure 2), and above 16.1% moisture for the sterile, inoculated sample. The normal seed was contaminated with both *Aspergillus flavus* and *A. glaucus* and the latter fungus proliferated more freely during respiration trials. The lower critical moisture value for the normal seed suggests that *A.*

glaucus has a lower humidity requirement than *A. flavus.* This is confirmed by the data of Koehler (1938) who showed that the minimum moisture requirement for the growth of *A. glaucus* on shelled corn was 14%, while that for *A. flavus* was 18%. Similarly, Galloway (1935) found that spores of *A. glaucus* would germinate in the presence of nutrients at relative humidities as low as 75%, while spores of *A. flavus* required 85% relative humidity. These humidity values correspond to equilibrium moisture levels of 14.1% and 18.0% in soybeans, according to the data of Ramstad and Geddes (1942).

The values for the initial and final moistures of the samples in this trial confirm the previous observation that mold growth and respiration cause moisture accumulation in the seeds. The inoculated samples which underwent no respiratory increases, as well as the high moisture control sample, showed no significant moisture changes.

Table VI indicates that no significant difference exists in the respiratory quotient of a mold when respiring at different moisture levels on a biologically inert substrate. This is in contrast to the respiratory characteristics of the same sample of normal seed where both seed and mold respiration are involved. The data show conclusively that the respiratory quotient of purely mold respiration is 1.0, under the conditions of this experiment, with soybeans. These facts lend confirmation to the hypothesis previously suggested that the respiration of the soybean seed itself is not only of a very low order of magnitude, but is also characterized by respiratory quotients well below unity. That this is the case was shown by a study of the respiratory behavior of a series of samples of the sound Wisconsin Manchu soybeans at moisture values below the critical. These data, presented in Table VIII, were obtained by using very large seed samples and low aeration rates, in order to obtain readily measurable changes in the composition of the respired air. It may be concluded that where mold growth on seeds

TABLE VIII

EFFECT OF MOISTURE CONTENT BELOW THE CRITICAL LEVEL ON RESPIRATORY RATE AND RESPIRATORY QUOTIENT

(High-quality Wisconsin Manchu soybeans on tenth day of trial at 30°C)

Moisture	Respiratory rate [1]	R.Q.
%		
9.3	0.11	0.50
11.3	0.21	0.56
12.4	0.34	0.67
13.4	0.41	0.73
14.3	0.56	0.76

[1] Milligrams CO_2 per 100 g dry matter per 24 hours.

is restricted by lack of air, high carbon dioxide concentration, or unfavorable moisture, the combined respiration of the seed and mold would yield a respiratory quotient intermediate between the low value due to the soybean seed and the value of 1.0 for purely mold respiration. Conversely, as mold respiration increases with increasing aeration and moisture, the predominant respiration of the molds forces the average respiratory quotient very close to unity.

Discussion

Two agencies are responsible for the respiration and deterioration of soybeans under practical storage conditions where insect life is not involved, namely the normal metabolic processes of the seed itself and the biological activity of molds, which are almost invariably present.

At moisture values below 14%, seed respiration only is involved, and changes in chemical composition and viability proceed at a very slow rate. The energy released by seed respiration is apparently too slight to promote the heating of seeds when stored in bulk. However, even in this moisture range, there is a loss in viability with increasing moisture, particularly when the seeds are stored at a relatively high temperature. The present studies confirm similar observations reported by Barton (1941) and Ramstad and Geddes (1942).

The slow decrease in germination which occurs on the storage of "dry" seeds at ordinary temperatures may possibly be associated with the extent of respiration, slight as it is under these conditions. The production of carbon dioxide is enhanced by increased moisture and temperature, and unless sufficient moisture is provided for germination, the accumulating carbon dioxide or other respiration products may reach levels toxic to the embryo. In this connection, Kidd (1916) has suggested that carbon dioxide acts as a narcotic to seeds thereby inducing dormancy, and that germination is related to a lowering of this inhibitory partial pressure of carbon dioxide in the tissues.

The present studies have yielded data which show that normal viable soybeans release carbon dioxide when wetted, or when their temperature is raised, over a period of a few days, before either normal seed or mold respiration becomes established. That this carbon dioxide release is probably not due to a high initial rate of respiration seems to be indicated by the fact that high respiratory quotients in the range of 1.5 to 2.0 appear during this process and fall to normal values when this "bound" carbon dioxide is dissipated. This observation is not in accord with the claim of Freitinger (1927) that dormant seeds have a continuing intramolecular type of respiration, owing to the low oxygen permeability of intact seed coats, until sufficient moisture is provided to swell and rupture the seed coat. Futhermore, Milner and Geddes (1945) demonstrated that very high sustained respiratory quotients indicative of anerobic respiration occurred in damp soybeans only when the seeds were maintained under nitrogen (containing 0.2% oxygen).

In the present studies, normal dormant soybeans respiring in air over extended time intervals exhibited an increasing trend of respiratory quotient between 0.50 and 0.76 in a moisture range between 9.3 and 14.3% (Table VIII). Meyer and Rader (1936) have shown that germinating soybeans, which doubtless respire at a considerably higher rate than "dry" dormant soybeans, exhibit a respiratory quotient of 0.65. Under the same conditions, the respiratory quotient of germinating wheat was 1.0. These observations suggest that dormant soybean respiration is of a normal aerobic type, but that substrate materials other than carbohydrates, possibly fats, are respired by soybeans. Another interpretation of the regular increase in respiratory quotient as the moisture approaches that favoring mold growth is a binding of respired carbon dioxide by the tissues in the face of otherwise normal aerobic respiration, the degree of retention of carbon dioxide decreasing with increasing moisture.

When the moisture content exceeds a minimum value (usually about 14% but dependent on a number of factors), a very sharp increase in the respiratory rate of soybeans occurs; this indicates that an additional and very active agency contributing to the respiration has come into play. The evidence is quite conclusive that molds are responsible. The present studies have shown that their respiratory quotients are very close to a value of 1.0, indicating that as far as the principal species encountered in these studies are concerned (*Aspergillus flavus* and *A. glaucus*), aerobic carbohydrate utilization is the major process.

The minimum moisture content of soybeans where the sharp upturn in respiration has been found to occur, namely 14%, corresponds to an equilibrium relative humidity in the surrounding atmosphere of approximately 75%. This humidity level is the minimum critical humidity at which the most xerophytic mold species proliferate. Walter (1931) has stressed the fact that various microfloral species have different minimal moisture requirements for growth, and Galloway (1935) has classified a large number of common molds of the genus *Aspergillus* and *Penicillium* on the basis of their minimal humidity requirements for germination and growth on nutrient media. In general, the *Aspergilli* include the most xerophytic of all mold species. Most of the members of this group which have been studied initiate growth in the presence of nutrients in the range of humidities from 75% to 85% in time intervals up to two weeks. The *Penicillia* appear to require a minimum humidity of 85%, while other species including *Rhizopus*, *Trichoderma*, and *Cladosporium* require minimum relative humidities of about 90% for spore germination. Bacterial spore germination and growth is not likely to be involved under normal conditions at relative humidities below 95%. In the present studies it was noted that the proliferation of *Aspergillus glaucus* on soybean seeds occurred at a moisture level some 3% lower than was required for the growth of *A. flavus*.

That the relative humidity of the atmosphere surrounding natural products rather than the hygroscopic moisture values appears to govern mold spore germination and growth is clearly indicated when the data of several investigators are correlated. Haines (1937) emphasizes this point in a review on the causes of the microbiological deterioration of meat. Lea (1933) found that lipolysis due to mold growth commenced on bacon fat at 75% relative humidity. Macara (1943), after investigation of the causes of mold growth on dried meat, found that on meat containing 40% fat and 10% water, the moisture noted being in equilibrium with a relative humidity of 74-75%, molds might grow after a few weeks. Rozsa (1935) in a review of the work of Pap (unpublished) concluded that a relative humidity of 75% initiated mold growth with resulting increases in oil acidity in stored wheat. Smith (1938) has provided an excellent analogy to explain these facts with the statement: "If dry samples of pure wool and pure cotton are exposed to the same (humid) atmosphere, the wool will take up approximately twice as much moisture as the cotton, and, leaving out differences due to chemical composition, the two samples will be approximately equally liable to mildew. . . ." Since the completion of the present studies, Snow, Crichton, and Wright (1944) have demonstrated that extremely wide variations in equilibrium hygroscopic moisture exist in different natural feedstuffs at common relative humidities. Their data confirm the conclusion that mold growth is determined by the humidity factor rather than the actual moisture content of the material.

A conclusion of fundamental importance is that on as diversified a range of natural products as wheat, bacon fat, meats, leather, textiles, flour, feeding stuffs, and book-binding materials, the minimal relative humidity at which certain mold spores, commonly contaminating such materials, may grow, is virtually the same. In other words, a narrow humidity range with a minimum of about 75% corresponds to the different "critical" moisture levels shown by a variety of natural products, and it is this humidity which is associated with the initiation of mold growth on all. Rozsa (1935) expresses this thought as follows: "The source (of moisture for the growth of microorganisms on wheat) is mostly the vapor content of the atmosphere of the storeroom, or the air layers wrapping wet kernels of wheat which are so rich in vapor. Not the actual moisture is responsible for the damage of the different mill products, as the danger occurs at very different moisture contents. The damage begins at identical relative vapor pressures--otherwise at the biological equivalent of the moisture content."

The critical humidity level (75%) at which mold growth is initiated is probably in hygroscopic equilibrium with the different moisture levels in various seed species (e.g., flaxseed 10.5%, wheat 14.5%) where upward respiratory trends, similar to those shown in the present studies with soybeans, have been reported. The data presented have shown that if inhibitory interseed carbon dioxide concentrations are avoided, the well-defined upward trend in respiratory rate at the critical humidity for mold growth occurs more sharply than previous reports have suggested. In order to determine the "safe" moisture limits for a grain whose storage characteristics are unknown, it had been the practice in the past to determine its respiration rate at various moisture levels and then to designate as "critical" that moisture value at which the sharp upward trend in respiration commences. That this laborious and time-consuming procedure is unnecessary is evident from the discussion given on the relationship of relative humidity to mold growth. With certain reservations to take into account factors due to seed condition, one need only determine at ordinary temperatures, the relative humidity-hygroscopicity moisture relationships of any material in order to designate as "critical" that moisture value which is in equilibrium with a relative humidity of 75%.

Bailey (1940) questioned the suggestion of Rozsa (1935) that mold growth can occur at relative humidities as low as 75%, and pointed out that even if this were possible, the equilibrium moisture content of wheat at this humidity, according to the data of Coleman and Fellows (1925), is 17 to 17.5%, which is well above the critical moisture in the range of 14 to 15% where the sharp upward break in the respiratory activity of wheat was shown to occur by Bailey and Gurjar (1918). On this basis, Bailey concluded that molds would not proliferate until the moisture content of wheat was in excess of 17.5%, and that phenomena other than mold growth are responsible for the rapid increase in wheat respiration in the critical moisture range. Bailey (1940) failed to note that the moisture data of Coleman and Fellows (1925) are expressed on the dry weight basis, and not on the conventional "as is" basis. When recalculated, their values show that 75% relative humidity is in equilibrium with a moisture content of 14.6% and not 17.5%. Their results then fall into close agreement with the hygroscopic moisture data for wheat published by Rozsa (1935). This, figure, 14.6%, incidentally, coincides with the minimum of the moisture range which Bailey and Gurjar (1918) termed critical for wheat, and, as has been pointed out, also agrees with published data on the minimum relative humidities at which certain members of the *Aspergillus* species of molds will germinate and grow.

The critical humidity at which mold spores will germinate and yield mycelial growth is affected by various factors the most important of which are time, temperature, and nutrient availability. Thus, McHargue (1920) noted that molds did not appear on whole corn until a moisture of 15% was reached, whereas mold growth developed on corn meal at moistures as low as 12%. Wilson (1928) has pointed out that mold growth on soybeans appears first in the cracks and broken places of the seed coats. Tomkins (1929) found that the latent period of germination of mold spores decreases with increasing availability of nutrients. Increased nutrients also increase the range of humidity and temperature over which spores will germinate and grow. Tomkins concluded that "when any one of the conditions is modified so as to favor growth, that is, make it more rapid, the limitation of the growth rate by variation of any other condition is rendered more difficult." Similar conclusions were reached by Groom and Panisset (1933) after a thorough study of the causes of mildewing of book materials. Barton-Wright and Tomkins (1940) found essentially the same relationships to hold true for the growth of molds in wheat flour. The critical importance of the time factor has been stressed by Snow, Crichton, and Wright (1944) who point out that while 75% relative humidity may be considered as a safe humidity for storage of most feedstuffs for time intervals up to three months, they were nevertheless able to detect spore germination and some mycelial growth in feeds after three years of storage at humidities as low as 65%.

The present studies have shown that the latent period of mold spore germination, corresponding to the time interval preceding rapid respiratory increases in damp soybeans, decreases with increasing moisture content. Furthermore, frost-damaged seeds show considerably shorter lag periods before the occurrence of respiratory increases due to mold growth, as well as much higher maximum respiratory potentials than did sound, high-quality seeds at comparable moisture levels. Frost-damaged seeds also showed somewhat lower critical moisture levels. These differences can be ascribed directly to the greater concentration and availability of mold nutrients in the damaged seeds, as well as to a greater degree of initial mold contamination. In this connection, Milner, Warshowsky, Tervet, and Geddes (1943) have shown that frost damage in soybeans is accompanied by increases in phosphate acidity, nonprotein nitrogen, and reducing sugars. In general, it would appear that the greater storage hazards encountered with immature grain, or grain damaged by mechanical injury, by freezing, sprouting, or weathering, as compared to well-matured grain at similar moisture values, can be ascribed to the fact that such grain provides a more favorable medium for mold growth than does normally matured, carefully harvested grain.

An interesting conclusion to be drawn from the data reported in the present studies, as well as that from the literature which has been reviewed, is that, of the large variety of microfloral species which usually contaminate normal soybeans, only a small group of common molds (certain species of *Aspergillus* of which *A. glaucus* is of greatest importance, and a very few *Penicillia*) are involved in the respiration and storage deterioration of soybean seeds within the moisture ranges normally encountered. It is notable that certain members of the *Aspergilli*, particularly *A. flavus*, which have been considered to be saprophytes, preferring dead organic media for growth, can grow on normal seeds and adversely affect their germination. That the metabolic activity of molds on stored grain may lead to marked increases in the moisture content of the seeds is clear from the data of this study. This is indicative that the mycelial mat which appears on moldy seeds is considerably more hygroscopic than are the seeds themselves, at the same relative humidity.

Summary

The influence of moisture content on the respiratory characteristics of soybeans of varying commercial quality at 37.8°C was studied by a technique providing for the simultaneous measurement of.oxygen consumption and carbon dioxide production, under conditions of continuous and controlled aeration, for time intervals up to 15 days.

Moisture values below about 14% yielded very low and virtually constant respiratory rates over extended time intervals, indicative of purely seed respiration. Small increments of moisture beyond this value were accompanied by respiratory increases over a period of several days until equilibrium respiratory conditions were approached. This effect was due to mold growth, and the plotted respiration values thus assumed the form of a microbiological growth curve. Accordingly with time, before equilibrium values were reached, increasingly sharper inflection of the respiration curve in the crtical moisture range was noted.

Latent period of mold spore germination, corresponding to time intervals preceding rapid respiratory increases decreased with increasing moisture content. Frost-damaged seedsshowed shorter respiratory lag periods and considerably higher repiration rates than did

high-quality soybeans at similar moisture levels, as well as a significantly lower critical moisture value than sound, well-matured seeds. These differences are ascribed primarily to the greater concentration and ease of availability of nutrients for mold growth in damaged as compared with sound seeds.

Respiratory characteristics of autoclave-sterilized (biologically inert) soybeans at various moisture levels, inoculated with spores of a mold indigenous to normal soybeans, showed the same characteristic sharp upward trend in respiration at a critical moisture value as did normal seeds; the low respiration values yielded by normal seeds at lower moisture values (seed respiration) were absent.

Wetting or heating of normal soybeans caused an initial evolution of carbon dioxide apparently not related to respiration, which is attributed to the release of preformed carbon dioxide bound by the dormant seeds.

Respiratory quotients increased with moisture content and approached 1.0 as the ratio of mold to seed respiration increased. When seed respiration was absent (autoclaved, mold-inoculated seeds), the respiratory quotient of 1.0 exhibited by the mold respiration was unaffected by moisture content.

Viability of soybeans decreased somewhat when the seed was held at 37.8°C at moisture contents below that favoring mold growth, but was most adversely affected at moisture contents where molds grew readily.

Only slight changes with time in the chemical composition of respiring soybeans as estimated by oil acid value and total and reducing sugars were noted at moisture values unfavorable to mold growth. At moisture levels where molds proliferated, drastic chemical changes occurred, approximately proportional to the moisture content and the extent of mold growth.

Significant increases in the moisture content of soybeans maintained in atmospheres of constant humidity in equilibrium with the seed moisture were noted when mold growth was extensive. At the same relative humidity, mold mycelia are probably more hygroscopic than are soybeans.

Aspergillus glaucus and *A. flavus* were the principal microflora which proliferated on soybeans in the course of respiration trials, in the moisture range studied. *A. glaucus* was the most xerophytic species encountered, appearing to initiate growth at about 14% moisture in soybeans (in time intervals within two weeks) corresponding to a relative humidity of 7[illegible]% in the surrounding atmosphere. *A. flavus* required a moisture value some 3% higher for germination.

The literature reviewed indicates that only a few mold types are capable of germination and growth at relative humidities as low as 75%, whereas an increasing diversity of microfloral species appears at higher humidities.

Relative humidity rather than actual moisture content of seeds determines their susceptibility to molding. It is concluded that the various "critical" moisture values for different seed species (e.g., flaxseed 10.5%, wheat 14.5%) are those moisture contents which are in hygroscopic equilibrium with a common relative humidity of about 75% which, over moderate time intervals, is the minimal humidity required for the growth of the most xerophytic mold species which contaminates the seed.

Acknowledgment

The authors are indebted to Mr. E. A. Andrews and Dr. C. M. Christensen of the Division of Plant Pathology, University of Minnesota, who provided the mycological data, and to Mr. Leo Holman of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, at Urbana, Illinois, who supplied the series of naturally moist Illini soybeans.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods (Fourth Ed.). American Association of Cereal Chemists, Lincoln, Neb.
Bailey, C. H.
1921 Respiration of shelled corn. Minn. Agr. Expt. Sta. Tech. Bull. 3.
1940 Respiration of cereal grains and flaxseed. Plant Physiol. 15: 257–274.
—— and Gurjar, A. M.
1918 Respiration of stored wheat. J. Agr. Research 12: 685–713.
Barton, L. V.
1941 Relation of certain air temperatures and humidities to the viability of seeds. Contrib. Boyce Thompson Inst. 12: 85–102.
Barton-Wright, E. C., and Tomkins, R. G.
1940 The moisture content and growth of mold in flour, bran, and middlings. Cereal Chem. 17: 332–342.
Cohn, F.
1890 Ueber Warme Erzeugung durch Schimmelpilze und Bakterien. Jahresber. Schles. Gesell. (Breslau) 68: 23–29.
Coleman, D. A., and Fellows, H. C.
1925 Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different relative humidity. Cereal Chem. 2: 275–287.
Darsie, M. L., Elliot, C., and Peirce, G. J.
1914 A study of the germinating power of seeds. Bot. Gaz. 58: 101–136.
Freitinger, G.
1927 Untersuchungen über die Kohlensaüre gabe und Sauerstoffaufnahme bei keimenden Samen. Flora 22: 167–201.
Galloway, L. D.
1935 The moisture requirements of mold fungi, with special reference to mildew in textiles. J. Textile Inst. (Transactions) 26: T123–129.
Gane, R.
1935 The respiration and water content of seeds. Dept. Sci. Ind. Research (Brit.). Report of Food Invest. Board pp. 135–137.
Gilman, J. C., and Barron, D. H.
1930 Effect of molds on temperature of stored grain. Plant Physiol. 5: 565–573.
Groom, P., and Panisset, T.
1933 Studies on *Penicillium chrysogenum* Thom, in relation to temperature and relative humidity of the air. Ann. Applied Biol. 20: 633–660.

Haines, R. B.
1937 Microbiology in the preservation of animal tissues. Dept. Sci. Ind. Research (Brit.) Food Invest. Board Special Report No. 45.

Kidd, F.
1916 The controlling influence of carbon dioxide. Part III. The retarding effect of carbon dioxide on respiration. Proc. Roy. Soc. (London) **B89**: 136–156.

Koehler, B.
1938 Fungus growth in shelled corn as affected by moisture. J. Agr. Research **56**: 291–307.

Kolkwitz, R.
1901 Ueber die Athmung ruhender Samen. Ber. deut. botan. Ges. **19**: 285–287.

Kretovitch, V. L., and Uschakova, E. N.
1940 Uber die kritische Feuchtigkeit und den Atmungsgaswechsel des Kornes beim Aufbewahren. Compt. Rend. Acad. Sci. U.R.S.S. (Doklady) **29**: 115–119.

Larmour, R. K., Clayton, J. S., and Wrenshall, C. L.
1935 A study of the respiration and heating of damp wheat. Can. J. Research **12**: 627–645.

——, Sallans, H. R., and Craig, B. M.
1944 Respiration of whole and dehulled sunflowerseed and of flaxseed. Can. J. Research **F22**: 9–18.

Lea, C. H.
1933 Chemical changes in the fat of frozen and chilled meat. Part V. The effect of smoking and the influence of atmospheric humidity on the keeping properties of bacon. J. Soc. Chem. Ind. **52**: 57T-63T.

Leach, W.
1944 Studies on the metabolism of cereal grains. III. The influence of atmospheric humidity and mould infection on the carbon dioxide output of wheat. Can. J. Research **C22**: 150-161.

Macara, T. J. R.
1943 Dried meat. II. The growth of molds on dried meat. J. Soc. Chem. Ind. **62**: 104–106.

McHargue, J. S.
1920 The cause of deterioration and spoiling of corn and corn meal. Ind. Eng. Chem. **12**: 257–262.

Meyer, B. S., and Rader, D. S.
1936 Simple apparatus for the quantitative determination of photosynthetic and respiratory ratios. Plant Physiol. **11**: 437–443.

Milner, M., and Geddes, W. F.
1945 Grain storage studies. II. The effect of aeration, temperature, and time on the respiration of soybeans containing excessive moisture. Cereal Chem. **22**: 484–501.

——, Warshowsky, B., Tervet, I. W., and Geddes, W. F.
1943 The viability, chemical composition and internal microflora of frost damaged soybeans. Oil and Soap **20**: 265-268.

Ramstad, P. E., and Geddes, W. F.
1942 The respiration and storage behavior of soybeans. Minn. Agr. Expt. Sta. Tech. Bull. 156.

Rozsa, T. A.
1935. Drinking habits of the wheat berry. Northwestern Miller, Production Annual **184** (2): 73–74.

Smith, G.
1938 An Introduction to Industrial Mycology. Edward Arnold and Co. Ltd., London.

Snow, D., Crichton, M. H. G., and Wright, N. C.
1944 Mould deterioration of feeding stuffs in relation to humidity of storage. Part I. The growth of moulds at low humidities. Ann. Applied Biol. **31**: 102–110.

1944 Mould deterioration of feeding stuffs in relation to humidity of storage. Part II. The water uptake of feeding stuffs at different humidities. Ann. Applied Biol. **31**: 111–116.

Swanson, C. O.
1934 Some factors involved in damage to wheat quality. Cereal Chem. **11**: 173–199.

Tervet, I. W.
1945 The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopathology **35**: 3-15.
Thom, C., and LeFevre, E.
1921 Flora of cornmeal. J. Agr. Research **22**: 179-188.
Thomas, R. C.
1937 The role of certain fungi in the sick wheat problem. Ohio Agr. Expt. Sta. Bimonthly Bull. 22 No. 185: 43-45.
Tomkins, R. G.
1929 Studies on the growth of molds. Proc. Roy. Soc. **B105**: 375-401.
Walter, H.
1931 Die Hydratur der Pflanze und ihre physiologisch-ökologische bedeutung. G. Fisher, Jena.
Wilson, H. K.
1928 Wheat, soybean, and oat germination studies with particular reference to temperature relationships. J. Am. Soc. Agron. **20**: 599-619.
Zeleny, L., and Coleman, D. A.
1938 Acidity in cereals and cereal products, its determination and significance. Cereal Chem. **15**: 580-595.

A STUDY OF THE MILL STREAMS COMPOSING 80% EXTRACTION FLOUR WITH PARTICULAR REFERENCE TO THEIR NUTRIENT CONTENT

C. R. Jones and T. Moran

Research Association of British Flour-Millers,
Cereals Research Station, St. Albans, England

(Received for publication February 19, 1946)

The composition in terms of the B vitamins, thiamine, riboflavin, and nicotinic acid of individual mill streams in white flour milling has been studied by Jackson, Doherty, and Malone (1943). The particular interest of the present study is that it represents a survey of the streams of a commercial mill making 80% National flour of relatively high vitamin content despite relatively short surface.[1] Thus it covers the adjustments and devices aiming at securing a substantial entry of the germ, particularly the scutellum, into the flour as a whole, which are now typical of British practice. These features were discussed by Kent, Simpson, Jones, and Moran (1944); also they are well summarized by J. F. Lockwood (1945). They will be described briefly in the present paper.

Milling Procedure

The tests were made with the cooperation of the Coxes Lock Milling Company Ltd., Weybridge, Surrey, a country mill with a capacity of 20 sacks (of 280 lb.) of flour per hour, i.e., in American terms, 686 barrels per 24 hours.

At the time of the test the grist was composed of 60% Nos. 1 and 2 Manitoba and 40% English wheat. The extraction actually obtained was 80.5% of National flour. This was calculated on total products with screenings and wheat cleanings running automatically through a grinder into the offal. The straight run or National flour was better than average in color, its color score (Moran, 1944) being about 10 as against a national average of 15, with a thiamine value above average (0.89 as against a national average of 0.80), and was of good baking quality.

The mill has four breaks (the fifth, put into operation when milling 85% flour, was abandoned on changing to 80% extraction), but the last three have coarse and fine subdivisions. The total break roll surface was only 26 inches per sack of 280 lbs. flour per hour, the total reduction roll surface 48, and *X* and *Y* 6, the total being 80, or only 70% of that regarded as typical of British white flour milling.

---

[1] In the United Kingdom, percentage extraction is calculated on total products including screenings. It is, therefore, about 2% higher than if calculated on clean dry wheat.

British and American Milling. In the following account wherever possible the equivalent American milling terms have been inserted in brackets after the corresponding English terms. It should be noted, however, that there is no general American or Canadian equivalent to the English scratch system—including *X* and *Y* rolls. This may be briefly explained in American terms as follows: The throughs ("brown sizings") of the last sheets of the first and second purifiers are sent to *X* roll which is fluted with 28 corrugations per inch and driven at 2½ : 1 differential. The tails ("chunks") of these purifiers, and also of the third purifier, are sent to a similar roll called *Y*. *Y* also receives the coarsest and most branny parts of the grind from *X*—as separated by *X* scalper and by *X* middlings purifier; the other parts pass to the early middlings reductions. The coarser part of *Y* grind is scalped to offal but its middlings are purified (*Y* purifier) and contribute respectively to the various coarse or tailings rolls. Thus instead of chunks being sent *direct* to tailings rolls (or IV *Bk* rolls) as in American practice they pass first to this intermediate-scratch-system.

Two other points of difference should be noted:

(1) Except for *A* purifier (purifying scalpings from first middlings coarse roll) it is not usual in British practice for reduction stocks to be purified. Thus, although there is a succession of purifiers operating on grades of break middlings, there is no exact equivalent to the American fourth purifier which may receive, as part of its feed, stock from the first tailings roll.

(2) The coarsest scalpings from the earlier coarse rolls invariably pass, in British practice, direct to the next coarse roll (e.g., B_2 tails to *F*, *F* tails to *J*), and not to offal as often in American practice, where only the second scalpings may be directed to the next coarse roll. This is important in the present discussion.

It should also be noted that it is difficult to equate exactly to British practice the fourth middlings roll, the feed to which appears generally to correspond partly with that to *E* and partly with that to *G* roll. We have bracketed *E* and *G* with the fourth middlings roll and put *H* as corresponding to the fifth middlings roll.

Operation of Mill. The principle (generally recognized in milling 80% flour) is followed at the Coxes Lock Mill of making very substantial releases early in both break and reduction systems, making as much semolina (coarse middlings) as possible, so that the roll surface allocation is in fact short for the early breaks and reductions but relatively long in the later stages. The later stages can therefore be used for careful selective action in the way of "cleaning up"; this is an important point. In the case of the reduction system this is helped by careful but heavy purification of well-graded semolinas (sizings) and middlings, which enables heavy flouring to be done easily by the head reduction rolls. Scalping and grading for all breaks except the fourth, and sifting of *A*, *B*, and *C* reduction stocks (stocks from reduction of first and second middlings) is by Speight plansifter. *B* dunst, though good, is redressed on a centrifugal with advantage. The remaining reduction stocks and those from the fourth break are handled on centrifugals with covers ranging from 10 to 13 silk. There is a conventional *X* and *Y* scratch system with *X* and *Y* purifiers. *A* roll tails are purified. Tray separations, and in some cases tails, from middlings and fine semo purifiers, and from *X* and *A* purifiers, are dusted in a centrifugal, affording a small quantity of "TINS" flour, before passing to tins purifier, which grades stock to *E*, *F*, and *K* (fourth middlings, first tailings, and sixth middlings) rolls, respectively.

The mill is normally equipped throughout. It has 13 reductions (to *M*) of which B_2, *F*, and *J* ("second quality" or fine tailings, first tailings, and second tailings, respectively) are the customary second, third, and fourth quality coarse reductions (using the convenient terminology of J. F. Lockwood's discussion of the Reduction System in *Flour Milling*, 1945). *D* and *E* (third and fourth middlings) are fine rolls following *C* and B_2; *G* and *H* (fourth and fifth middlings) are the next group of fine rolls, following *F;* while *K*, *L*, and *M* (sixth, seventh, and eighth middlings or first low grade) are successive fine rolls following *J*. There are no detachers. Material from the last break, after treatment in the IV *Bk* purifier, passes along with that from *Y* purifier to each of the coarse rolls, B_2, *F*, and *J*. The IV *Bk* dunst goes to *K*, as also does "Tins" dunst. The only reductions having fluted rolls at the time of the test were *J* (the lowest grade coarse roll) and *L*, each of which had 40 flutes per inch and 2½:1 differential. It should be noted that *L* also received pollards (approximately "shorts": second scalpings from the last break); in that way *L* and *M* include the functions of a Z_1 and Z_2.

Analytical Methods

The quantities of the individual flours liberated by each of these stages in the milling process (expressed as percentages of the wheat) are shown in the second column of Table I. These are based upon timings made by the mill staff. The last 10 columns of this table show the results of chemical analyses using the following methods:

Ash was determined by incinerating a 5 g sample overnight in a silica vessel at 600°C and the resulting ash weighed.

Fiber was determined by the method officially used for National flour in Great Britain as recommended by the Analytical Methods Committee of the Society of Public Analysts (1943).

TABLE I

COMPOSITION OF INDIVIDUAL MILL STREAMS CORRESPONDING TO NATIONAL FLOUR (80.5% EXTRACTION)
(Arranged in order of decreasing flour color; all results expressed on a 13% moisture basis)

Stream		Yield[2]	Protein (N×5.7)	Fat[3]	Fiber	Ash	Thiamine	Riboflavin	Nicotinic acid	Iron	Manganese	Total phosphorus	Notes on flour. Color based on wetted Pekar slide
Description	American equivalent[1]												
		%	%	%	%	%	µg/g	µg/g	µg/g	mg/100 g	mg/100 g	%	
Group I. The Creamy Group (Patent Flour)													
A	Flour from 1st middlings coarse roll	10.4	10.7	0.93	0.10	0.40	0.54	0.40	9.0	0.69	0.30	0.082	*A* to *D* inclusive had a very similar creamy color, almost free from specks.
B	1st middlings fine	16.9	11.1	0.94	0.10	0.43	0.57	0.37	9.7	0.62	0.33	0.086	
C	2nd middlings	15.2	11.0	0.89	0.10	0.43	0.57	0.40	10.7	0.52	0.34	0.087	
D	3rd middlings	3.5	10.8	1.15	0.10	0.52	1.08	0.33	12.0	0.67	0.44	0.108	
		46.0											
B^1	2nd quality roll or fine tailings roll	2.6	10.1	1.84	0.10	0.66	2.34	0.56	15.0	1.19	0.81	0.138	Slightly darker and greyer than above, color turns pale brown on wetting. Slightly specky.
Group II. The Greyish-Cream Group (Appreciably Specky). Just Slightly Better than National Flour													
Subgroup (A)													
E	Part of 4th middlings	2.0	11.7	1.23	0.10	0.60	1.74	0.53	13.5	1.00	0.64	0.125	*E* to *FMD* inclusive were distinctly more specky than those in group I but color was still creamy rather than greyish.
II *Bk*		3.5	14.0	1.25	0.18	0.63	1.02	0.60	18.0	1.10	0.54	0.131	
CMD[4]	Break middlings duster	3.5	12.4	1.29	0.15	0.65	1.68	0.68	19.4	1.18	0.59	0.132	
FMD[5]		2.9	12.4	1.28	0.20	0.66	1.53	0.61	13.5	1.18	0.62	0.133	
		11.9											
Subgroup (B)													
G	Part of 4th middlings	3.5	11.9	1.70	0.15	0.67	2.40	0.60	17.2	1.30	0.88	0.145	*G* to I *Bk* inclusive were slightly less creamy (greyer) and more specky than those in Subgroup A.
X		0.2	11.5	1.94	0.17	0.77	2.97	0.70	25.5	1.68	0.94	0.165	
I *Bk*		3.2	11.6	0.88	0.19	0.57	0.54	0.52	18.0	1.00	0.44	0.112	
		6.9											
National Flour (80.5% straight run) Pivotal line			12.0	1.43	0.202	0.724	2.67	0.69	19.2	1.41	0.99	0.154	

TABLE I—(*Continued*)

Stream: Description	Stream: American equivalent[1]	Yield[2]	Protein (Nx5.7)	Fat[3]	Fiber	Ash	Thiamine	Riboflavin	Nicotinic acid	Iron	Manganese	Total phosphorus	Notes on flour. Color based on wetted Pekar slide
		%	%	%	%	%	μg/g	μg/g	μg/g	mg/100 g	mg/100 g	%	
Group III. Just Slightly Poorer in Color than National Flour													
Tins		0.2	11.7	1.46	0.37	0.74	1.65	0.64	24.6	1.84	0.72	0.145	Rather grey-brown but not very specky.
Group IV. The Brown Group													
Subgroup (A)													
F	1st tailings roll	1.2	12.3	2.45	0.24	0.97	4.05	0.92	25.1	2.19	1.55	0.211	*F* and III *Bk* were relatively dark, yellowish and specky. Color turns quite brown on wetting.
H	5th middlings roll	2.2	13.2	2.07	0.33	0.97	4.11	0.92	24.0	2.15	1.79	0.214	
III *Bk*		0.8	16.5	1.77	0.35	0.95	1.83	0.80	64.0	1.94	0.93	0.203	
		4.2											
Subgroup (B)													
Y		2.7	12.8	2.75	0.36	1.33	5.64	1.10	50.0	3.16	1.82	0.296	*Y* and IV *Bk* were rather darker but less coarsely specky than *F* and III *Bk*. Both exceptionally greasy.
IV *Bk*		0.9	16.0	2.54	0.41	1.82	2.85	1.44	84.0	4.04	1.59	0.400	
		3.6											
Group V. The Dark-Brown Group													
J	2nd tailings	1.4	17.7	5.35	0.97	3.24	32.40	4.00	32.9	8.50	8.27	0.730	All flours in this group were much speckier, dirtier, and browner than those in any of the above groups. *M* dunst redresser was much the worse; *K* was rather paler than the others.
K	6th middlings	1.2	15.6	3.65	0.90	2.17	16.20	2.60	56.5	5.53	4.96	0.483	
L	7th middlings	1.3	17.3	4.37	1.01	3.15	19.80	3.20	101.0	7.97	6.48	0.705	
M	8th middlings or 1st low grade	0.6	17.9	4.93	1.29	3.34	22.50	3.25	133.0	8.45	7.94	0.752	
M dunst redresser	2nd low grade	0.6	19.2	5.85	2.38	3.65	25.80	3.55	111.0	9.65	10.90	0.813	
		5.1											
Offals													
Coarse	Bran	7.1	12.4	3.86	10.85	5.78	4.95	2.89	296.0	11.80	12.20	1.172	
Fine		12.4	14.3	4.69	8.30	4.57	10.38	3.40	188.0	12.20	13.45	0.926	
Calculated weighted average of offals		19.5	13.6	4.39	9.23	5.00	8.40	3.21	227.0	12.1	13.00	1.014	
Calculated weighted average of all products		100.0	12.31	2.01	1.97	1.56	3.81	1.18	59.8	3.49	3.34	0.322	
Wheat (determined values)		100.0	12.37	2.03	1.97	1.51	3.87	1.28	57.2	3.14	3.41	0.321	

---

[1] The American equivalent term is given where different from English.
[2] Expressed as a percentage of the wheat represented.
[3] Petroleum ether extract.
[4] Coarse middlings dresser flour.
[5] Fine middlings dresser flour.

Fat was determined by extraction under a reflux condenser with boiling petroleum ether (B.P. 40° 60°C).

Thiamine was determined as recommended by Nicholls, Booth, Kent-Jones, Amos, and Ward (1942) and Booth (1942).

Riboflavin assays were conducted according to the method of Barton-Wright and Booth (1943).

Nicotinic Acid was determined by the method of Barton-Wright (1944).

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, A.O.A.C. (1940) and connected to protein by the factor 5.7.

Iron was determined by a method based on the work of Cowling and Benne (1942), Koenig and Johnson (1942), and Saywell and Cunningham (1937). The color of the ferrous o-phenanthroline complex was measured with a "Spekker" absorptiometer, using Ilford No. 604 filters and 4-cm. cells.

Manganese was determined as permanganate after oxidation with persulfate and silver nitrate. The method is based on the work of High (1945) and Richards (1930). Ilford No. 604 filters and 4-cm. cells were used.

Phosphorus was determined by the method of Allen (1940) with the substitution of 2 ml of $5N$ sulfuric acid solution for the 2 ml of 60% perchloric acid. Ilford No. 608 filters and 1-cm. cells were used.

The line next to the lowest in Table I shows the weighted average composition of all products, in comparison with the determined figures for the wheat set out in the bottom row. The comparison is clearer in Table II, which also shows the percentage deviations. The satisfactory nature of the sampling and timing (i.e., figures for percentage weights of the various streams) is shown by the fact that the deviations are spread each side of zero.

TABLE II

COMPARISON OF AVERAGE ANALYSES OF ALL PRODUCTS WITH VALUES DETERMINED ON WHEAT

Component	Determination on wheat	Weighted average of all products	Percentage difference [1]
Ash, %	1.51	1.56	+3.3
Fiber, %	1.97	1.97	0.0
Fat, %	2.03	2.01	−1.0
Thiomine, μg/g	3.87	3.81	−1.6
Riboflavin, μg/g	1.28	1.18	−7.8
Nicotinic acid, μg/g	57.2	59.8	+4.5
Protein, %	12.37	12.31	−0.5
Iron, mg/100 g	3.14	3.49	+11.1
Manganese, mg/100 g	3.41	3.34	−2.0
Total phosphorus, %	0.321	0.322	+0.3

[1] Calculated on the values for wheat.

Iron Content of Flour

Having regard to the standards of accuracy in the various analytical methods the only serious, or, rather, significant, deviation is that of iron. This figure is known to be considerably affected through contact of materials with iron machinery, and it is conceivable, though perhaps surprising, that the 11% increase in iron content, from wheat to products, may be due to entry of iron particles and dust during processing. At first sight the chilled iron rollers of a flour mill might be expected to contribute to the iron content of roller-milled flour, since their working life is by no means indefinite.

According to Mr. J. F. Lockwood [2] of Messrs. Henry Simon, Ltd., in mills running 6,000 hours annually, fluted rolls were changed on the average every two years before the war, because wear on the edges of the teeth had altered the shape of the flutes. Rolls in mills with long surfaces might last 50% longer, short surfaces considerably less. Flute depths in common use vary between 0.0182 and 0.009 inch. No great increase in rate of wear has occurred under war conditions, except that reduction rolls fluted 35 and 40 per inch have required more frequent refluting, as they have frequently been set up in contact. Even in these cases the reduction in the roll diameter during work is not more than 3 or 4 thousandths of an inch per year. Reduction rolls with smooth surfaces run for very many years with a wear of less than three thousandths of an inch.

Assuming that the wear of a smooth reduction roll is 0.003 inch per annum (three one-thousandths in 10 years) it may be calculated that, for a reduction roll surface of 60 inches per sack, the liberation of metallic iron might raise the iron content of the total flour made by 0.007 mg per 100 g. It is rather more difficult to calculate a probable contribution from fluted rolls, but assuming that 0.0025 inch is actually worn off every flute per year, the contribution of iron from a total break roll surface of 40 inches per sack to the total flour milled would be only 0.0013 mg per 100 g.

Scrapers used on smooth rolls wear appreciably. In the experience of Mr. T. S. Parker, of Messrs. Henry Simon, Ltd., the wear amounts to $\frac{3}{8}$ inch in 20 years. Such scrapers average about $\frac{3}{32}$ inch in thickness. On this basis the contribution of metallic iron to the total flour milled would be only 0.0002 mg per 100 g, for 60 inches of smooth roll surface per sack.

The total contribution of metallic iron from all rolling operations is therefore unlikely to exceed 0.01 mg per 100 g. This is less than one thirtieth of the difference actually found in the present series between the wheat and the total products. The difference actually found, viz., 0.53 mg per 100 g, on the basis of 7000 lbs. per hour of wheat milled (as in the mill under discussion) would amount to $\frac{1}{30}$ lb. per hour of metallic iron or say ½ lb. per day, or 1½ cwt. per year. This seems large, but can hardly be ruled out as impossible, since much wear must take place in worm conveyors, elevators, spouts, and cleaning machines. It is these rather than the actual roller mills that would seem to be the main contributors of metallic iron to the flour and offal.

---

[2] Private communication.

It is also conceivable that the discrepancy originates from fragments of iron, entering with the grain, which pass into the screenings. These might be missed in sampling the wheat but would be reintroduced to the Fine Offal with the ground screenings.

Color and Nutrient Content of Streams

The main commercial consideration in flour has always been that of color and in Table I the various flours have been arranged in that respect. "Color," as discussed here, is that of the *wetted Pekar slide*, as customarily used in commercial practice, and not the "color score," which has proved so useful in classifying long-extraction (straight-run) flours. The latter is a measure of content of discrete bran specks, and is applied to straight-run flours from different mills. It is not directly applicable to the present series, where the individual streams vary greatly in degree of fineness of the contained bran. The order of arrangement in Table I is quite familiar to the miller, who ordinarily looks to the head reduction rolls, *A*, *B*, and *C*, to furnish half the total output of flour, in the form of patent flour: that is, the flour of creamiest and cleanest color (freest from bran specks). The next group of flours listed adds just over a third, making altogether 84%, of the total output of flour, and has color appreciably and progressively poorer (more greyish, more specky) than the patent group, but none of its members is poorer than the National, or 80% straight run, flour (see the "Pivotal Line" in Table I). Below this line lie the "brown" and the "dark-brown" groups, making about 10% and 6% respectively of the total flour output. Of these the dark-brown group in entirety would have entered the weatings or offals in prewar white flour milling, while some of the brown group would not have been quite so brown, principally because less severe work would have been done in the earlier parts of the milling system. Moreover, for the same reason, the upper groups themselves would have been fractionally better in color and lower in ash content. The top three flours (*A*, *B*, and *C*; making up about half the total flour) are, as a group, from 0.05% to 0.1% higher in ash than corresponding flours from ordinary prewar (70% extraction) milling. Most of the remaining flours, down to

Group 3 in Table I, are higher in ash, by between 0.1 and 0.2%, than corresponding prewar flours. Thus a flour corresponding to 70% of the wheat, made by excluding the darkest 10.5% of the flour streams of Table I, should by calculation contain 0.49% ash, which is almost 0.1% higher than that of a good prewar 70% flour. A 70% flour actually made a little later in this way, by excluding the bottom 10% of the National flour, gave the following analytical figures:

Ash	0.51%
Thiamine	1.05 μg/g
Fiber	0.15%

Table I shows how well the ash content corresponds with the relative placing of the flours according to color. This is not surprising, for ash content has been long recognized as an effective means of characterizing various grades of flour in commerce. Only two flours are really out of place in the double ranking (i.e., according to color *and* to ash content), viz. B_2, roll flour and the first break flour. B_2 is exceptional in being the first of the tailings or lower grade coarse rolls, i.e., it operates on a feed containing a fair amount of germ which has been flattened in the preceding higher grade reductions. B_2 roll flour therefore is the first flour to contain an appreciable proportion of germ. At the same time, its speckiness or bran content is slight because the feed to B_2 roll, though germy, is not very branny—hence the rise in thiamine, fat, riboflavin, nicotinic acid, and ash, etc. (in all of which the germ is more or less rich)—while the fiber content (reflecting bran content) is not appreciably increased. The first break flour is opposite in that it has lower ash content than its appearance would indicate. This flour contains substantially no germ, as indicated by the very low thiamine figure, but does contain an appreciable amount of bran powder—shown by the rise in the fiber figure, which, incidentally, is in the right place in the ascending order of the Table.

Table I shows that, in general, the content of nutrients rises with falling color (and also with falling baking quality, discussed in a later section). The dark-brown group as a whole is much the richest in nutrients. In certain general characteristics the trend accords with that found by Jackson, Doherty, and Malone (1943) in their analyses of the mill streams of a large Canadian mill, which was milling all-Manitoba wheat to a flour extraction of 74%. Their riboflavin and nicotinic acid values for the wheat itself were very similar to those of the present wheat mixture, but the thiamine content was higher (4.53 as against 3.87 μg/g). The ash content of their various grades of flour, up to a total extraction of, say, 70%, ran lower than the present values substantially in the manner described above. Despite this, their nicotinic acid values for the patent flour (top 37% of wheat), for the next grade (first baker's, bringing the total length to 63%) and for the third grade (second baker's, to 70%) were, respectively, very similar to those shown in Table I of this article for corresponding groups of flours. On the other hand their thiamine and riboflavin figures were both relatively higher in the top (patent) grade but lower (thiamine, considerably; riboflavin, slightly) in the two lower grades.

In Table I the flours from the tailings, or lower quality coarse, rolls show sudden steps upward in thiamine content—at B_2, *F*, and *J*—because these are points of concentration and re-entry of germ. With a system entirely of smooth rolls, the flours from the group of fine reductions immediately following a given coarse roll would be expected to show no downward trend in thiamine, because the dunsts from the coarse roll feeding them would also be rich in germ. Thus *H* flour is as high as *F* flour in thiamine content. Usually, however, there is dilution of the feed to such fine reductions with fresh material from the break system or from earlier fine reductions. For example, *G* also receives fine middlings from the early breaks and dunst from *E*, which lower the nutrient content of its flour (and as every milling student knows, improve its appearance or grade). *K* is similarly affected, relatively to *J*, by dilution with dunst from the fourth break and from *H*. When, however, a low-grade coarse roll is fluted (cf. *J* roll in the present series), its flour shows a markedly high thiamine figure, owing to the greatly increased breakdown of germ at that point.

The break flours generally are very low in thiamine content relative to their color position and (possibly apart from the last break flour) contain practically no germ. Their high content of certain nutrients is due to their progressively increasing content of bran (as reflected in the fiber figure) and, more important, of endosperm from the peripheral portions of the grain. This endosperm has been shown (Moran, 1945) to be high in iron, nicotinic acid, and protein. This explains the fluctuations in the nicotinic acid figures in Table I. Outer endosperm enters, progressively more markedly, into the II, III, and IV break flours, and to some extent (as would be expected since scratch rolls resemble fine break rolls) into the scratch roll flours *X* and *Y*. It also affects the nicotinic acid value of *L* flour because, as mentioned, this roll receives pollards ("shorts" or fine bran), and it appears that, possibly partly for this reason, the last reduction, or *M* and *M* redresser, flours contain considerable proportions of outer endosperm. The nicotinic acid content of bran itself is relatively very high and this itself accounts in part for the nicotinic acid levels at the points mentioned.

The level of protein content is interesting in that it is affected in two ways. The outer endosperm is relatively high in protein content and the germ is exceptionally high (over 30%). The first factor accounts for the quite high level (16-16.5%) reached in the third and fourth break flours, while the second accounts for the very high level of the very germy flours, e.g., *J* (17-18%). When outer endosperm appears to be present as well as germ, the figure becomes higher still, as in *M* redresser flour (19.2%).

Riboflavin appears on the whole to be well related to ash content (and to order of color).

The fat data deserve consideration especially in connection with those for thiamine. To begin with it appears that the outer endosperm must be very high in fat content. This must be assumed, in order to account for the relatively high fat contents of the later break flours which contain little or no germ. It is again in keeping with the high figure (the highest fat content shown in Table I) for the *M* redresser flour, already assumed to contain outer endosperm.

Germ itself contains at least 10% of fat (extracted with petroleum ether). Table I indicates that the main or inner endosperm from this grist, in relatively pure condition, has a fat content of about 0.9%. One would expect therefore the excess over 0.9% of the fat content of any given flour to be connected with the proportion of germ contained in it, and therefore to its thiamine content. So far, however, nothing has been said about the two subdivisions of the germ, viz., the scutellum and embryo. In this paper the term "embryo" will later be used, purely for convenience, to denote what should strictly be termed "the part of the embryo other than scutellum" (i.e., the dormant root, stem, and plumule). As shown by Hinton (1943, 1944) the former is the main seat of thiamine in grain, having a thiamine value of about 150 μg/g while the embryo has only about 9 μg/g. It is probable that the scutellum contains more fat than the embryo (Hinton, 1944), but the fat content of both is high. Any flour which contains much more scutellum than embryo should therefore have a relatively high ratio of thiamine to fat, and vice versa. It is interesting to inspect Figure 1, which shows the thiamine values for the various samples of Table I, plotted against fat contents. Only in a general sense does thiamine rise with fat content, as shown roughly by the straight line drawn cutting the horizontal axis, as seems natural at about 0.9%. Points for some flours lie well above and some below this line. It is suggested that the former flours contain a more or less high ratio of scutellum to embryo, and the latter a low ratio. The outstanding case, of course, is *J* where the ratio of scutellum to embryo must be particularly high. It is significant that *J* is the first *fluted* roll in the reduction system of this mill. This confirms the value of finely fluted rolls for reducing scutellum, as stressed by Kent *et al.* (1944). The graph also indicates that each of the flours from the tail end of the reduction system (*K* and onwards) is rich in scutellum relative to embryo, while the reverse is the case with the germy flours from earlier in the system, e.g., B_2 and *F*. It would be very interesting to see where the point in the graph for *F* flour would lie in a similar mill having fluted *F* rolls.

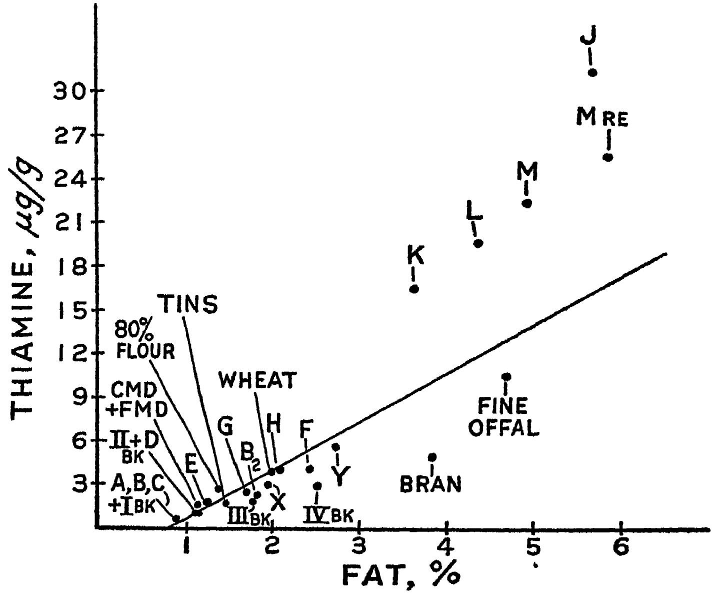


Fig. 1. Relation between the thiamine and fat content of flour streams composing National flour of 80.5% extraction.

The phosphorus and iron content follows the ash content closely (Table I). Manganese, however, follows the germ content more particularly, which is to be expected, since W. J. S. Pringle in these laboratories finds that the manganese content of the grain is relatively highly concentrated in the germ. Thus in the present data though the proportion of manganese is less than half the iron in the pure endosperm (e.g., *A* flour), yet it tends to reach or even to exceed the figure for iron in the germy flours.

Baking Tests

Baking tests were made employing absorptions determined with the instrument developed by Halton and Fisher (1937). These absorptions (calculated to a 14% moisture basis) and the heights of the 1-lb. loaves baked are shown in Table III. The system used 4 hours'

TABLE III

RESULTS OF BAKING TESTS ON INDIVIDUAL FLOURS COMPRISING NATIONAL FLOUR OF 80.5% EXTRACTION
(Arranged in order of decreasing crumb color)

Flour	Baking tests without potassium bromate				Baking tests with potassium bromate			
	Water[1] absorption	Loaf[3] height	Dough quality	Crumb color	15 ppm. $KBrO_3$ added general loaf quality	Bromate tolerance		Notes on response
						Total bromate	Loaf height[2]	
	%	Inches				ppm.	Inches	
				Group I. Pale Cream Crumb				
A	58.6	4.9	All flours in this group yielded good doughs with fairly good to good extensibility and spring.	*A* and *C* gave similar pale cream crumb colors. *B*, *D*, and *E* gave progressively slightly deeper color.	*A* and *C* excellent with close even grain and smooth texture. *B*, *D*, and *E* very like above but crumb slightly less regular. *B* had slightly firmer crumb than others.	15		
C	59.6	5.0				15		
B	57.8	5.0				15		
D	64.3	4.9				15		
E	65.3	5.1				15		
				Group II. Deep Cream Crumb				
B_2	59.6	4.4	Generally good doughs with good extensibility and spring and with but slight stickiness except B_2 and *X*. B_2 gave a very sticky, slightly short dough with only fair spring. *X* gave a very sticky dough. I *Bk*, II *Bk*, *CMD*, and *FMD* gave very extensible doughs.	Progressively slightly deeper color with greyish-brown tinge becoming more noticeable, *G*, *X*, and Tins being considerably darker than the others. Even B_2 distinctly deeper and more brownish than *E* in top group.	B_2 to II *Bk* inclusive alike in crumb which was rather less even but firmer and less well developed and with a rougher texture than group I.	20	4.6	Only slight increase in loaf volume. I *Bk* to II *Bk* inclusive gave large volume increase with slightly open and irregular crumb, but of good spring.
I *Bk*	55.7	4.6				20	5.2	
FMD	57.5	4.5				20	5.2	
CMD	56.5	4.6				20	5.2	
II *Bk*	58.2	4.7				20	5.2	
G	62.5	4.6		*G* to Tins gave softer and better developed crumbs than B_2 to II *Bk* but of poorer color.		20	5.2	Large volume increase, very soft crumb of good spring.
X	56.8	4.4				20	4.8	Slight volume increase, bromate addition gave better grain and spring of crumb.
Tins	58.2	4.9				20	4.9	No response. crumb rather weak.

TABLE III—(*Continued*)

	Baking tests without potassium bromate				Baking tests with potassium bromate			
						Bromate tolerance		
Flour	Water[1] absorption	Loaf[2] height	Dough quality	Crumb color	15 ppm. $KBrO_3$ added general loaf quality	Total bromate	Loaf height[2]	Notes on response
	%	*Inches*				*ppm.*	*Inches*	
				Group III. Pale Brown Crumb				
III *Bk*	60.0	4.4	All doughs were very extensible but sticky. All gave good spring except *H*. III *Bk* gave particularly good spring.	III *Bk* and *F* similar (pale brown).	III *Bk* to *H* gave smaller loaves with denser crumb than group II. *H* rather more open and softer than III *Bk* and *F*.	25–35	5.4	Marked volume increase; well-developed soft but rather irregular crumb.
F	61.5	4.5				25–35	4.7	Some volume increase, better developed; crumb much improved including color.
H	71.5	4.3		*H* slightly browner and *Y* and IV *Bk* markedly browner.		25–35	4.7	Some volume increase, better developed; crumb much improved including color.
Y	56.8	3.9			*Y* and IV *Bk* gave still smaller and denser loaves.	25–35	4.5	Great improvement in volume and development. Crumb improved but still close in grain.
IV *Bk*	61.5	3.8				25–35	5.2	Outstanding improvement in all characters.
				Group IV. Dark Brown Crumb				
K	66.1	2.8	Very unsatisfactory doughs, progressively very short, sticky and clay-like.	Progressively poorer color from *K* to *MRE* (*MRE*, a dirty grey-brown).	All flours gave very small, bound and dense loaves.	35–55	2.8	All flours in this group gave little volume increase but there was some improvement in the crumb.
L	64.3	2.8				35–55	2.8	
J	62.9	2.4				35–55	2.8	
M	67.9	2.4				35–55	2.8	
MRE	84.3	2.4				35–55	2.8	
National Flour	61.8	5.0	Good extensibility and spring, slightly sticky.	Flour was bleached; gave a greyish-white crumb color.	Larger loaf and more open and woolly than groups I to IV inclusive.			

[1] Expressed on 14% moisture basis.

[2] Height of 1 lb. loaf (tinned).

fermentation (including final proof) at 80°F (26.7°C), with 1.3% yeast and 1.8% salt. The doughs were made and molded by hand throughout.

The samples are arranged in Table III according to the color of the loaf crumb. They fall into four broad groups (the second of which consists of two subgroups), well differentiated from one another. Group 1, with pale cream color, and Group 2, with deep cream crumb color, substantially reproduce the order of the first two groups of Table I (which was based upon flour color), except that (1) *E* flour comes up into the top group and B_2 moves down to the second group; (2) the order of the I and II *Bk* flours is interchanged; (3) the tins flour is brought up into Group 2. Group 3, in Table III, corresponds with the brown group of Table I; the III *Bk* flour, however, comes out at the top of this group on the score of crumb color. Group 4 includes members of the dark-brown group of Table I, none of which makes anything approaching acceptable bread when baked alone.

The last three columns in Table III are of special interest as showing the different responses to improver treatment of these various components of the National flour. The first two groups require no more than light treatment and of their members the early break flours (and middlings dresser flours, which are really break flour), *G* and *X* show considerable response. The third group as a whole requires heavy treatment and responds markedly (the fourth break flour, as is well known, being outstanding in its response to improver treatment). The members of the fourth or dark-brown group show slight improvement and require very heavy dosage.

This work has been carried out on a mixed grist. Though no consideration of possible segregation of the components (Manitoba and English) in the milling process has been attempted, it is believed that the differences recorded are due mainly to the interplay of the different parts of the grain in the ways discussed rather than to segregation of particles from hard and soft wheats.

Summary

Proportions and composition (protein, ash, fiber, fat, thiamine, riboflavin, nicotinic acid, iron, manganese, and phosphorus) are given for each of the 23 individual flours which compose a National flour of 80% extraction and good color, and for the corresponding offals. The weighted averages agree well with the corresponding figures for the wheat (60% Manitoba, 40% English), except in the case of iron, where the amount in total products exceeds that in the wheat by 11% of the value; considerations of known extents of wear indicated that this

entry of iron must have occurred in a part of the plant or process other than the actual roller-mills.

The special measures generally adopted in milling 80% flour in Britain are briefly described, regard being given to differences between American and English technical terms. The streams composing the best (or "patent") half of the 80% flour have 0.1% fiber content and ash content not exceeding 0.43%: this ash content is from 0.05 to 0.1% higher than that of corresponding flours from ordinary 70% extraction milling. The difference becomes greater with the lower grade flour streams.

The ash and fiber contents of the flour streams in general accorded well with the placing according to color. The best 12 of the streams, amounting to 84% of the total flour, were all superior or equal to the National or 80% straight-run flour in color and, generally, lower or not higher in content of ash, fiber, B vitamins, iron, manganese, and phosphorus. When untreated or with only light improver treatment, these flours had the best baking quality. The remaining flour streams fell substantially into two groups, the brown and dark-brown, comprising 10% and 6% respectively of the total flour, with markedly rising contents of ash, fiber, and all nutrients. The latter group, much the richer in nutrients, would in 70% extraction milling pass entirely to offal while some members of the brown group would have been less brown. Members of the dark-brown group, individually, have very poor baking quality, though in the total flour they are "carried" by the general strength of the grist. They show very great tolerance to improvers, as exemplified by bromate, though the response is disappointingly small. Tolerance is less, though still great, in the brown group but here the response is marked.

The thiamine values for the series of streams show marked increases in the flours from the tailings, or low-quality coarse, rolls because these represent points of concentration of germ. These increases are particularly marked when such rolls are fluted (in accordance with present general British practice); for example, in the present series the *J*, or second tailings, roll was the first fluted reduction roll and the flour sifted from its grind (*J* flour), amounting to 1.4% of the wheat, had a thiamine content exceeding 30 μg/g.

The break flours contain little or no germ. Their relatively high content of some nutrients, especially nicotinic acid—progressively greater in approaching the later breaks—is due to increasing content of bran powder, and, more important, of outer endosperm. This, while not particularly rich in thiamine, is very rich in nicotinic acid, protein, iron, and fat. The later break flours are thus high in protein and in fat because they contain much outer endosperm, while the later reduction flours are high in protein and fat because they contain much germ. In the last reduction (second low-grade) flour of the present mill, both germ and outer endosperm are present and protein and fat both reach maximum values.

In this series of flours the relationship between fat and thiamine content is not a close one. This is to be expected, not only from the disturbing effect of the outer endosperm (rich in fat but not in thiamine), but also because thiamine is mostly concentrated in the scutellum, while both scutellum and nonscutellum parts of the embryo are rich in fat. The latter enters preferentially into some of the flour streams whereas in others, particularly towards the end of the reduction system, the scutellum enters preferentially.

Any or each of the three—germ, outer endosperm, and bran—may contribute to the ash content of the individual mill streams. The riboflavin, phosphorus, and iron values follow the ash contents, but the manganese values follow more particularly the contents of germ.

Literature Cited

A.O.A.C.
1940 Official and tentative methods of analysis of the Association of Official Agricultural Chemists. 5th ed. Sect. II, para. 23.
Allen, R. J. L.
1940 The estimation of phosphorus. Biochem. J. **34**: 858–865.
Analytical Methods Committee
1943 Determination of the crude fibre in National flour. Analyst **68**: 276–278.
Barton-Wright, E. C.
1944 The microbiological assay of nicotinic acid in cereals and other products. Biochem. J. **38**: 314–319.
——, and Booth, R. G.
1943 The assay of riboflavin in cereals and other products. Biochem. J. **37**: 25–30.
Booth, R. G.
1942 Vitamin B_1 assay in national wheatmeal. Analyst **67**: 162.
Cowling, H., and Benne, E. J.
1942 Report on zinc and iron in plants. J. Assoc. Official Agr. Chem. **25**: 555–567.
Halton, P., and Fisher, E. A.
1937 An improved process for determining the baking qualities of wheaten flour dough and apparatus therefor. Brit. Patent 492,049, March 23, 1937.
High, J. H.
1945 The photometric analysis of copper-base alloys. II. The determination of manganese by oxidation at room temperature. Analyst **70**: 18–19.
Hinton, J. J. C.
1943 A micro-method for the estimation of vitamin B_1. Biochem. J. **37**: 585–589.
1944 The chemistry of wheat germ with particular reference to the scutellum. Biochem. J. **38**: 214–217.
Jackson, S. H., Doherty, A., and Malone, V.
1943 The recovery of the B vitamins in the milling of wheat. Cereal Chem. **20**: 551–559.
Kent, N. L., Simpson, A. G., Jones, C. R., and Moran, T.
1944 High vitamin flour. Ministry of Food Publication. Also in Milling **103**: 294–300.
Koenig, R. A., and Johnson, C. R.
1942 Spectrophotometric determination of iron. II. Use of 2,2′-Bipyridine. J. Biol. Chem. **143**: 159–163.

Lockwood, J. F.
1945 Flour Milling, pp. 413–415. The Northern Publishing Co. Ltd., Liverpool.
Moran, T.
1944 The colour of 82.5 per cent National flour. Milling **103**: 300.
1945 Nutrients in wheat endosperm. Nature **155**: 205–206.
Nicholls, J. R., Booth, R. G., Kent-Jones, D. W., Amos, A. J., and Ward, H. H.
1942 Vitamin B_1 (aneurine) assay in white flour. Analyst **67**: 15–17.
Richards, M. B.
1930 The colorimetric determination of manganese in biological material. Analyst **55**: 554–560.
Saywell, L. G., and Cunningham, B. B.
1937 Determination of iron. Ind. Eng. Chem. (Anal. Ed.) **9**: 67–69.

USE OF SPRAY-DRIED WHOLE-EGG POWDER IN SPONGE CAKES[1]

RUTH JORDAN and MARGARET S. PETTIJOHN

Department of Home Economics, Agricultural Experiment Station,
Purdue University, Lafayette, Indiana

(Received for publication January 24, 1946)

The purpose of this investigation, one of a series on the use of spray-dried whole-egg powder in cookery, was to study the leavening property of dried eggs and to develop a practicable method, if possible, for making acceptable sponge cakes from the whole-egg powder.

In sponge cakes, eggs serve as a leavening agent by incorporating and retaining air when beaten. They exert a binding effect, serving to maintain the cell structure of the batter during the baking process, and thus helping to impart lightness and characteristic texture to the cake. They also give flavor and color to the cake (Bennion, Hawthorne, and Bate-Smith, 1942).

The leavening power of eggs depends not only upon the amount of air which can be incorporated during whipping, but also upon the strength of the foam produced (Hawthorne and Bennion, 1942). A foam of suitable stability, if deftly handled while the flour is being incorporated, allows the air to remain in the batter during the final mixing and baking, and the resulting cake will be light, tender, and of good volume (Pyke and Johnson, 1940). If the foam is not sufficiently strong to support the flour, the finished cake will likely be less tender and have smaller volume (Hawthorne and Bennion, 1942).

Since the age and quality of eggs, the temperature of beating, and other factors affect the rate at which egg-sugar foams or meringues are formed when eggs and sugar are beaten together, it has been suggested that specific gravity determinations and not time should be used to indicate when a batter is beaten sufficiently if cakes of high quality and uniformity are to be obtained (Pyke, 1941; Pyke and Johnson, 1940, 1941).

---

[1] Journal Paper No. 243, Purdue University Agricultural Experiment Station.

A few reports on the whipping and baking properties of dried whole-egg powder have appeared in the literature. Bennion, Hawthorne, and Bate-Smith (1942) found a correlation between the volume of the egg-sugar foam and the quality of the sponge cakes baked from the foam. They reported that, in general appearance and volume, cakes made with dried egg plus a secondary aerating agent were only slightly inferior to cakes made from frozen eggs. They concluded that by slight alterations made in usual baking procedures, good quality spray-dried whole-egg powder, properly prepared and stored, could be used to produce sponge cakes of medium to good quality, even without the addition of a second aerating agent. They observed that better results were obtained when a temperature of 100°F (37.8°C) was used for beating the egg-sugar-water mixture than when the beating was done at 65°-70°F (18.3°-21.1°C). Hawthorne and Bennion (1942) made a more extensive study of the influence of temperature upon the beating and baking properties of spray-dried egg. They found considerable improvement resulting from the use of higher temperatures than are usually employed in commercial practice. Both the volume of the foam and the rate of formation of the foam were increased by beating the reconstituted egg with sugar at the higher temperature. Using a temperature of 104°F (40°C), 5 to 10 minutes of beating yielded as large a volume of foam from a given egg sample as 20 to 30 minutes beating at 64°F (17.8°C). In some cases, still greater improvement in the formation of the foam resulted from using temperatures around 120°-122°F (48.9°-50°C) for whisking, provided additional water was added to the egg-sugar mixture before mixing to compensate for evaporation during beating. These investigators concluded that, by choosing suitable conditions, it should be possible to make first-quality sponge cakes from almost any sample of spray-dried egg, although some special equipment might be necessary in bake shops.

Woodcock and Reid (1943) used the volume of sponge cakes made from various egg powders as one measure (among many others) of assessing the quality of powders prepared under different drying conditions.

Reid and Pearce (1945) in a study of the relationship of various objective tests to baking quality of dried whole-egg powder found a high correlation ($r = + 0.96$) between foaming volume of the powders and loaf volume of small sponge cakes baked from the same powders, when prepared under the conditions of temperature and humidity used in their experiments.

Materials and Procedure

For the first exploratory work in which 83 cakes were baked, the egg powder used was spray-dried whole-egg powder of good quality which conformed to the 1944 specifications of the War Food Administration as to moisture content. For the remainder of the study, there was available low-moisture powder which was produced according to 1944 U. S. Army specifications. This low-moisture powder came packed in No 10 tin cans, and each was labeled with the date of drying. As soon as the egg powder was received in the laboratory, the date of receipt was recorded and the powder was placed at once in a refrigerator maintained at about 40°F (4.4°C). When a can of the powder was opened, the contents were placed in quart glass jars and tightly covered. The egg powder was kept in the refrigerator until used.

The other ingredients used in each series of cakes were as nearly identical as possible. The granulated sugar for all the cakes came from the same lot, as did also the salt; cake flour of the same brand was used throughout, and for any given series it came from the same lot. Tap-water was used for reconstituting the egg powder.

The temperature of the ingredients was controlled as much as feasible. Records were kept of the temperature of the laboratory, of the foam, and of the batter.

Objective measurements made included the specific gravity of the egg-sugar foam and of the finished batter; the weight and volume of each cake; and the tensile strength of slices from the cake. The volume was determined by seed displacement, using a method similar to that described by King, Morris, and Whiteman (1936). The tensile strength determinations were made with an apparatus similar in design to the one used by Pyke and Johnson (1940). Slices used for tensile strength measurements were cut from the center of the cake; each was cut 2 cm thick and trimmed and cut into an hour-glass shape, the width of the center of which was 2 cm. The tensile strength readings were recorded as grams of force required per square centimeter of cross-section area to break the slice of cake.

The pH of the batters was determined in one series of cakes, a Beckman pH meter with glass electrode being used.

The beating and mixing were done for the most part on an electric mixing machine, using a wire whisk and 4-quart bowl. In the exploratory work, an electric mixer with the blade type rotary beater was used, but, since the mixer with the wire whip seemed to produce a better foam and required less time for beating, it was used in all later experiments.

The cakes, except in a few special cases, were baked in pans

3⅝ × 5⅝ inches at the bottom, 4⅛ × 6 inches at the top, and 2½ inches deep. The weight of batter baked in each pan was 175 g. Thermostatically controlled, ventilated gas ovens were used for baking.

In the exploratory experiments, the effect of different proportions of flour and sugar (within ranges found in standard recipes for fresh egg sponge cakes) to a given weight of egg powder was noted; the effect of various initial temperatures of ingredients upon the speed of foam formation was studied; and the specific gravity of foam and of batter which produced the better cakes was observed. As a result of these observations the following formula was tentatively set up for use in more detailed studies: Cake flour, 40 g; granulated sugar, 70 g; whole egg powder, 26.2 g; salt, 0.5 g; cream of tartar, 0.5 g; water, 95 to 105 ml.

Series I. In this series of more than 120 cakes, the following variables were studied: Three levels of water (95, 100, and 105 ml); two baking temperatures (375°F [190.6°C] for 19 minutes, and 400°F [204.4°C] for 15 minutes); and five levels of cream of tartar (0.0, 0.5, 1.0, 1.5, and 2.0 g).

The relative quality of the cakes was judged organoleptically by observing the grain, lightness, moistness, and flavor of samples; and objectively by the measurements of volume and tensile strength. Analyses of variance of the data were made in order to determine the relative importance of the variables used in the formula and in the manipulation. The correlation coefficients between specific gravity of the finished cake and that of the foam and of the batter were calculated.

Having established, through the experiments in Series I, some of the conditions for making an acceptable sponge cake from egg powder, still further improvements were sought. A modified sponge cake formula, including baking powder (a cream of tartar baking powder), gave promise of some improvement. Hence, a series of cakes was baked studying this factor.

Series IIa. A factorial experiment was set up in which three levels of cream of tartar and three levels of baking powder (0.0, 0.5, and 1.0 g) were used in all combinations, making a total of nine formulas. Since it was not feasible to bake more than five cakes in one baking period, an incomplete block design was used for the experiment. Ten replications of each cake were made; there was a total of 18 blocks of five cakes each, and each cake was baked in a period with every other cake five times. The order of the blocks and the order of mixing cakes within a block were selected at random. Determinations of volume and of tensile strength of the baked cakes were made and the data analyzed for statistical significance.

The formulas and the method of mixing for this series of 90 cakes were as follows:

BASIC FORMULA FOR SPONGE CAKE

Flour, cake	40 g
Sugar, granulated	70 g
Dried egg powder, low-moisture	26.2 g
Salt	0.5 g
Water, tap	95 ml
Lemon extract, ¼ teaspoon	

The combinations of cream of tartar and baking powder were as follows:

Key to formula	Cream of tartar g	Baking powder g
B	0.0	0.0
C	0.0	0.5
D	0.0	1.0
E	0.5	0.0
F	0.5	0.5
G	0.5	1.0
H	1 0	0.0
A	1.0	0.5
I	1.0	1.0

The egg powder used for this series was dried in February, 1945, and used during March, April, and May following.

The procedure for mixing and baking was as follows:

(1) The sugar, egg powder, salt (and cream of tartar, if used) were sifted together into the mixing bowl which was placed in a pan of hot water, double-boiler fashion. These ingredients were mixed and stirred until warmed to a temperature of 104°F (40°C).

(2) The 95 ml of water at a temperature of 176°F (80°C) were added all at once to the dry ingredients in the bowl and the mixture stirred lightly. The temperature of the resulting mixture was near 140°F (60°C).

(3) The mixing bowl was at once attached to the electric mixer and whipped (using wire whip attachment) at high speed for 30 seconds. The flavoring was added, and the whipping continued at high speed until the meringue reached a specific gravity of 0.270 to 0.306. (The time necessary to reach this point varied from 3.5 to 7.5 minutes with an average of 4.87 minutes.) The temperature of the meringue at the end of the beating period was usually about 86°F (30°C).

(4) The flour (and baking powder, if used), sifted once, was added all at one time to the egg-sugar foam. The mixer was started at low speed, and the flour was mixed into the foam for a period of 30 seconds. The mixer was stopped, the bowl scraped down, and then the mixing continued at low speed for another half-minute. The mixing was such that the specific gravity of the batter was 0.342 to 0.378.

(5) The batter—175 g—was poured into an ungreased pan.

(6) The cake was baked for 19 minutes in a 375°F oven.

The cake was cooled and left in the inverted pan overnight. The following day the volume and tensile strength measurements were made.

Series IIb. When the pH meter became available for use, a series of 45 cakes was baked, five cakes being made by each of the nine formulas used in the series of 90 cakes. The pH of the batters was taken in order to note any relationship which might be apparent between pH of batter and quality of the cake. The egg powder used in this series was dried in April, 1945, and used in July following.

Supplementary Group. A group of miscellaneous cakes was baked in which ingredients were measured instead of weighed, and in which some cakes of family size were baked. Some fresh egg cakes were baked for comparison.

Results and Discussion

In the preliminary work, it was observed that if the temperature of the egg-sugar-water mixture was around 140°F (60°C) at the beginning of the beating period, a lighter and more stable foam was formed and a much shorter beating time was required than if the ingredients were at room temperature, 70°–77°F (21.1°–25°C). This observation was in agreement with the findings of Hawthorne and Bennion (1942).

The cakes in Figure 1 illustrate the effect of temperature and time of beating the foam upon the quality of the finished cake. Cakes 1, X, and Y were made with the same proportion of ingredients. The egg-sugar-water mixture for Cake 1 was at a temperature of 140°F (60°C) when beating was started and the time of beating required to produce the foam of satisfactory specific gravity was 5.5 minutes. For Cake X, the egg-sugar-water mixture was beaten at room temperature for 5.5 minutes, and, in that length of time, no satisfactory foam was produced; consequently, the cake was a failure. For Cake Y, the egg-sugar-water mixture was beaten at room temperature for 15 minutes, but, even after that length of time, the foam was not as light as for Cake 1 and, consequently, the resulting cake was not of as great volume. The formula for Cakes 1, X, and Y included 1.0 g each of cream of tartar and baking powder. The formula for Cake Z had 0.5 g cream of tartar, but no baking powder; the egg-sugar foam was beaten at room temperature for 25 minutes.

The effect of other variables in the formula and procedure was studied. From analyses of variance made on data from groups of cakes baked in Series I, using tensile strength measurements as the criterion of quality, it was found that the variance due to three levels of water—95, 100, and 105 ml—used in the formula, made no statistically significant difference in the cakes. The same was true of the two baking temperatures, 375°F (190.6°C) for 19 minutes and 400°F (204.4°C) for 15 minutes. The effect upon tensile strength of using different levels of cream of tartar, varying from 0.0 to 2.0 g, however, was highly significant (beyond the 1% level of significance). By organoleptic tests, cakes made with 95 ml water and 0.5 to 1.0 g cream of tartar in the formula, and baked at 375°F (190.6°C) for 19 minutes were generally judged to be preferable.

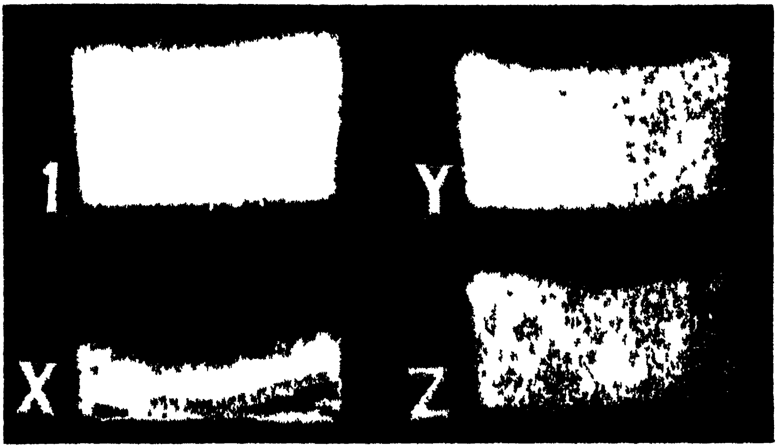


Fig 1 Effect of initial temperature of egg-sugar water mixture and time of beating upon quality of cakes made from dried egg powder. Cakes 1, X, and Y were made by the same formula which included 1.0 g each of cream of tartar and baking powder. The formula for Cake Z included 0.5 g of cream of tartar but no baking powder.

	Initial temperature of egg-sugar-water mixture °F °C	Time of beating the foam *min*
Cake 1	140–60.0	5.5
Cake X	75–23.9	5.5
Cake Y	75–23.9	15.0
Cake Z	75–23.9	25.0

The coefficients of correlation between the specific gravity of the baked cake and that of the egg-sugar foam or meringue, and that of the batter, were 0.63 and 0.77, respectively. These coefficients were based upon records from 138 cakes. Both of the values were statistically highly significant. A lower coefficient of correlation between batter and cake might be expected, since a light foam may not always be sufficiently strong to support the flour (Hawthorne and Bennion, 1942).

In Table I are given the averages of the measurements on the cake batters and finished cakes made in Series IIa and IIb. The 45 cakes in Series IIb were baked from a different lot of egg powder and under

TABLE I

AVERAGE RESULTS OF MEASUREMENTS ON THE CAKE BATTERS AND FINISHED CAKES OF SERIES II

Key symbol	Cream of tartar g	Batter: Specific gravity: Egg-sugar foam	Batter: Specific gravity: Batter	Batter: pH	Finished cake: Specific gravity	Finished cake: Specific volume[1] cc/g	Finished cake: Tensile strength[1] g/cm²
SERIES IIa—90 cakes							
0.0 g baking powder							
B	0.0	0.286	0.376	—	0.190	5.26	30.07
E	0.5	0.294	0.365	—	0.186	5.37	29.79
H	1.0	0.294	0.373	—	0.184	5.43	25.06
0.5 g baking powder							
C	0.0	0.297	0.372	—	0.182	5.49	28.92
F	0.5	0.297	0.366	—	0.180	5.56	28.25
A	1.0	0.294	0.359	—	0.179	5.58	24.59
1.0 g baking powder							
D	0.0	0.291	0.365	—	0.175	5.71	26.84
G	0.5	0.296	0.355	—	0.173	5.78	25.88
I	1.0	0.295	0.353	—	0.176	5.68	22.73
SERIES IIb—45 cakes							
0.0 g baking powder							
B	0.0	0.280	0.365	7.65	0.191	5.23	25.16
E	0.5	0.288	0.364	6.29	0.191	5.23	25.68
H	1.0	0.290	0.362	5.50	0.190	5.27	22.32
0.5 g baking powder							
C	0.0	0.278	0.352	7.38	0.185	5.40	23.95
F	0.5	0.277	0.345	6.40	0.182	5.49	23.25
A	1.0	0.283	0.352	5.64	0.185	5.40	21.45
1.0 g baking powder							
D	0.0	0.284	0.354	7.22	0.179	5.58	22.72
G	0.5	0.287	0.342	6.41	0.178	5.61	24.42
I	1.0	0.293	0.351	5.66	0.183	5.46	21.99

[1] Least difference in means necessary for significance in Series IIa (90 cakes):

	Specific volume	Tensile strength
At 5% level.	0.14	1.46
At 1% level	0.18	1.94

different laboratory conditions of temperature and humidity from the 90 cakes of Series IIa, yet the results were very similar.

Tensile strength measurements are an indication of relative tenderness; the lower the tensile strength, the more tender the cake. Cakes made from formulas A, H, and I, containing 1.0 g of cream of tartar, were the most tender both in Series IIa and IIb, as indicated by the lower tensile strength values. An analysis of variance on data from the 90 cakes indicated that both the cream of tartar and the baking powder influenced the tensile strength measurements to a highly significant degree, but the effect due to the cream of tartar was the more pronounced.[2] This is shown graphically in Figure 2.

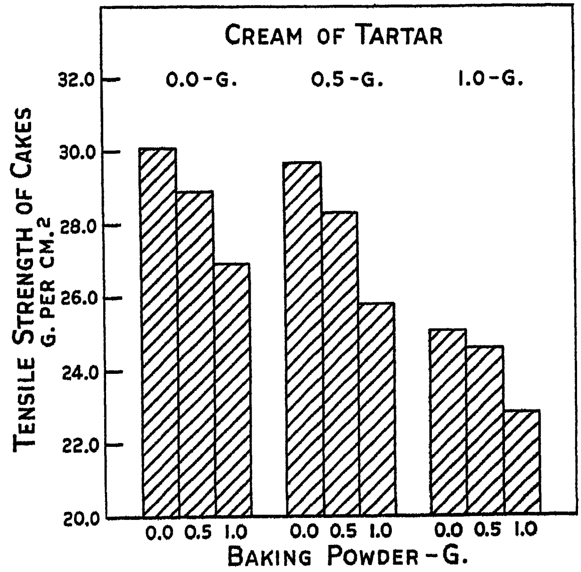


Fig 2. Effect of three levels of cream of tartar and of baking powder upon the tensile strength of sponge cakes made from spray-dried whole-egg powder.

The average volume of the cakes baked from 175 g of batter varied from 799 cc to 868 cc for the 90 cakes of Series IIa, and from 802 cc to 855 cc for the 45 cakes of Series IIb. The average specific volumes (volume in cc per gram of weight) of cakes from each formula are shown in Table I. The analysis of variance showed that the variation in volume due to cream of tartar was nonsignificant, but that due to the baking powder was highly significant.[3] The cakes baked from the formulas D, G, and I, containing 1.0 g baking powder, were of greatest volume. The effect of the two variables—cream of tartar and baking powder—is shown in Figure 3.

---

[2] The F value, or the ratio of the mean square of the variance of the treatment to the mean square for error, was 65.01 for the cream of tartar, and 26.65 for the baking powder. According to Snedecor's table of F values (Snedecor, 1940), the F value necessary for significance at the 5% level is 3.11; for the 1% level, 4.88.

[3] The F value for the mean square for the cream of tartar was 2.36, for the baking powder, 37.35. For values necessary for significance, see footnote 2.

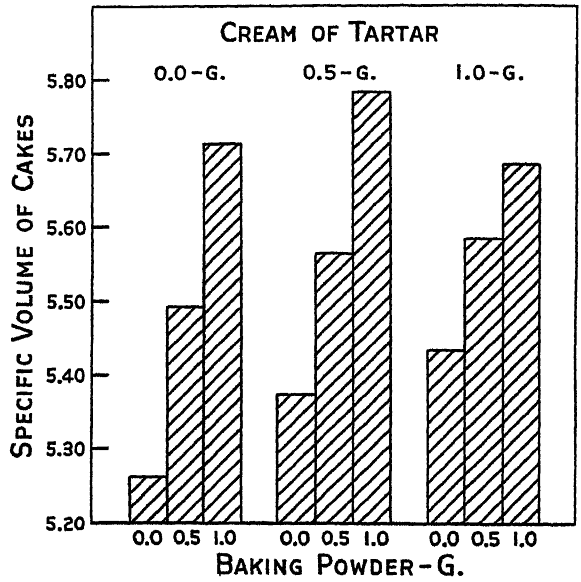


Fig. 3. Effect of three levels of cream of tartar and of baking powder upon the volume of sponge cakes made from spray-dried whole-egg powder. Specific volume is expressed as cc per g of weight.

The coefficient of correlation between the volume and the tensile strength was found to be −0.56, a value which was statistically highly significant, but which was not large enough to indicate that increase in volume is always accompanied by decrease in tensile strength (or increase in tenderness). For example, the cakes baked by formula G were of larger volume than the other cakes, but were not as tender as some of the cakes of smaller volume, and were not so desirable from the standpoint of fineness of grain.

Formula A, in which 1.0 g of cream of tartar and 0.5 g of baking powder were included, consistently gave cakes of fine grain, of good volume (though not the largest volume), and of acceptable tenderness (though not the greatest tenderness). Formula B, in which neither cream of tartar nor baking powder was included, usually produced the least tender and the smallest cakes. Formula I, in which 1.0 g each of cream of tartar and baking powder was used, yielded cakes which were usually slightly more tender and of slightly larger volume than the A cakes. Typical slices of cakes made from formulas A, B, and I are illustrated in Figure 4.

The pH values of the batters of the 45 cakes baked from the nine formulas ranged from 5.50 to 7.65 (see Table I). Batters containing no cream of tartar had average pH values ranging from 7.22 to 7.65; those containing 0.5 g cream of tartar, 6.29 to 6.41; and those containing 1.0 g cream of tartar, 5.50 to 5.64. The coefficient of correlation between the pH of the batters and the tensile strength of the finished cakes was found to be +0.34, a value significant at the 5% level. However, this low figure would indicate that other factors in addition to the pH of the batter tend to influence the tenderness of the cakes.

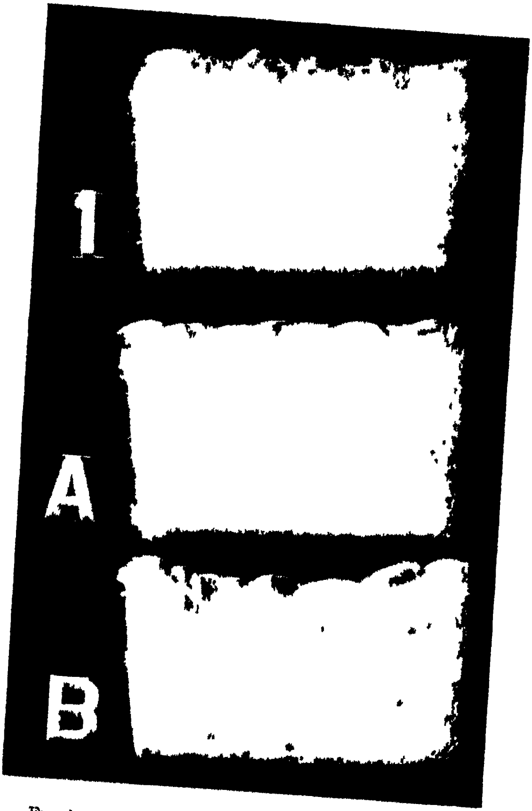


Fig 4. Typical cakes baked from formulas B, A, and I.

	Cream of tartar g	Baking powder g
Cake B	0.0	0.0
Cake A	1.0	0.5
Cake I	1.0	1.0

The specific gravity and the pH of the batters, and the specific volume and tensile strength of these cakes made from spray-dried whole-egg powder, compared favorably with corresponding measurements reported by King, Morris, and Whiteman (1936) and King, Whiteman, and Rose (1936) on cakes made from fresh eggs. The specific gravity of the batters and of the cakes also fell within the range advocated by Pyke and Johnson (1940) for sponge cakes baked at altitudes of 0 to 2500 feet

Typical of the family-size cakes made from the egg powder is the one shown in Figure 5. The formula used was three times the Formula I that was used in the small cakes and was equivalent to a six-egg sponge cake.

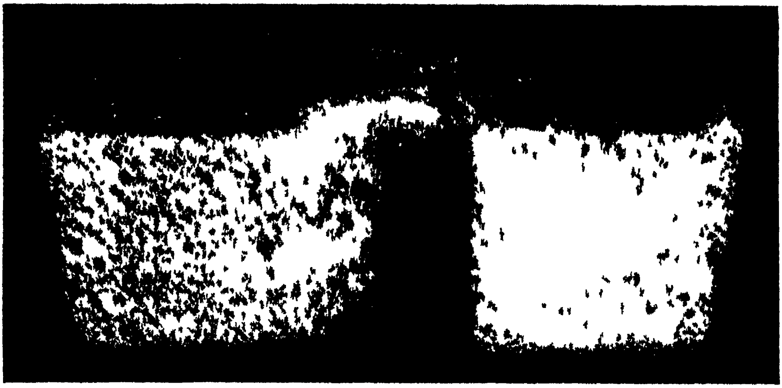


Fig 5 Typical family-size sponge cake baked from spray-dried whole-egg powder.

The time for beating the larger amount of egg-sugar-water mixture to a satisfactory foam was somewhat longer. It was found advantageous to add the flour gradually, rather than all at once. Baking the cake at 325°F (162.8°C) for 50 minutes gave satisfactory results.

Summary

The leavening property of spray-dried whole-egg powder was tested by using it for making sponge cakes. Acceptable cakes were made both with and without the addition of a secondary leavening agent (baking powder) to the formula.

Temperature was found to be an important factor in the production of a satisfactory egg-sugar foam or meringue from dried eggs. When the initial temperature of the egg-sugar-water mixture was 140°F (60°C), and beating was done on an electric mixer with a wire whip, a foam of suitable lightness for a small-size sponge cake was produced in 3.5 to 7.5 minutes of beating time.

Cream of tartar increased the tenderness of the cakes. The addition of a small amount of baking powder to the formula increased the volume somewhat and contributed to some extent to the tenderness of the cakes. Hence, a modified sponge cake formula in which some baking powder is included is recommended for use if dried eggs are used for sponge cakes.

Literature Cited

Bennion, E. B., Hawthorne, J. R., and Bate-Smith, E. C.
1942 Beating and baking properties of dried egg. J. Soc. Chem. Ind. **61**: 31–34.

Hawthorne, J. R., and Bennion, E. B.
1942 Influence of temperature on the beating and baking properties of spray-dried egg. J. Soc. Chem. Ind. **61**: 151–153.

King, F. B., Morris, H. P., and Whiteman, E. F.
1936 Some methods and apparatus used in measuring the quality of eggs for cake making. Cereal Chem. **13**: 37–49.

——, Whiteman, E. F., and Rose, W. G.
1936 Cake-making quality of eggs as related to some factors in egg production. Cereal Chem. **13**: 703–711.

Pyke, W. E.
1941 Factors the baker should consider in preparing the yellow sponge cake. Cereal Chem. **18**: 92–106.

—— and Johnson, G.
1940 Preparing and baking yellow sponge cake at different altitudes. Colo. Agr. Expt. Sta. Tech. Bul. 27.
1941 Relationships between certain physical measurements upon fresh and stored eggs and their behavior in the preparation and baking of cake. Poultry Sci. **20**: 125–138.

Reid, M., and Pearce, J. A.
1945 Dried whole egg powder. XVII. Objective tests and baking quality. Can. J. Research **23F**: 239–242.

Snedecor, G. W.
1940 Statistical Methods (3rd ed.). Collegiate Press, Inc., Ames, Iowa.

Woodcock, A. H., and Reid, M.
1943 Dried whole egg powder. IX. Effect of drying conditions on quality. Can. J. Research **21D**: 389–393.

CHEMICAL STATE OF PHOSPHORUS IN STARCH AS INDICATED BY TITRATION CURVES ON ELECTRODIALYZED STARCHES [1]

D. R. BRIGGS and MARTIN HANIG

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minnesota

(Received for publication November 29, 1945)

Starches from all sources, as ordinarily prepared, contain phosphorus in small but variable amounts. There is always an amount of phosphorus which cannot be removed from the starch by dialysis or extraction with aqueous solvents. The state of chemical combination of this phosphorus appears to vary with the plant source of the starch. Samec (1927) has been chiefly responsible for showing that in certain starches (e.g., potato starch) phosphorus, as phosphoric acid, is in ester combination with carbohydrate constituents of the starch, giving to the starch the properties of a colloid electrolyte. That this type of combination of starch with phosphorus may not exist for cereal starches is indicated by the fact (see Samec and Kleman, 1931) that these starches do not show the marked acidic properties, upon electrodialysis, that are characteristic of potato starch. Posternak (1935) showed that the phosphorus of potato starch, after acid hydrolysis, remained in combination with glucose, while the phosphorus of wheat and corn starches, after similar hydrolysis, was found to exist in combination with glycerol. Evans and Briggs (1941) found that methanol extraction of air-dry corn starch removed only about one-third of the total phosphorus while removing 95% of all lipid matter. The phosphorus removed was not identifiable as phospholipid. On the other hand, Schoch (1942) reported that practically all the phosphorus of wheat starch can be extracted with 80% dioxane and believes that most of the phosphorus in wheat starch is in phospholipid combination. Lehrman (1945) attained similar removal of phosphorus from wheat starch by a long-time extraction with methanol.

---

[1] Paper No. 2262, Scientific Journal Series, Minnesota Agricultural Experiment Station.

The present paper reports an attempt to learn something more about the state of the phosphorus in potato, corn, and wheat starches by a study of the form of the titration curves of the exhaustively electrodialyzed starches.

Materials and Methods

Starches. Six potato starches were studied, a commercial potato starch, PVI, and five samples prepared from varieties grown on fertilizer plots by the Division of Soils (University of Minnesota). Of the latter, PI and PV were from Cobbler potatoes grown on check and phosphorus-fertilized plots respectively, and PII, PIII, and PIV were from check plot plantings of Red Warba, Early Ohio, and Triumph varieties, respectively. Samples of the commercial potato starch were also methanol extracted (PVI, DF), and moisture heat-treated (PVI MH) according to the method of Sair and Fetzer (1944) before being electrodialyzed and their titration curves run.

Two samples of corn starch, a commercial product (C) and the same starch after defatting (CDF) by methanol extraction, were studied. These were the same starch samples which were studied by Evans and Briggs (1941).

Two wheat starches, a commercial starch (W) and the same after a methanol extraction (WDF), which was similar to that used in the preparation of corn starch (CDF), were employed in the study.

Electrodialysis of the Starches. Titration curves on colloid electrolytes can be dependable only when all diffusible electrolytes and counter ions (other than H^+ or OH^- ions) have been removed from the high molecular weight (or colloid) components. Often simple dialysis of the aqueous solution or suspension of the colloid against distilled water followed by an extended period of electrodialysis is able to accomplish this process without causing any other changes in the physical or chemical properties of the colloid. This simple procedure is not successful with starch for the following reasons. The phosphorus present in starch (e.g., potato starch), which cannot be removed by simple dialysis or extended washing of the starch granules with distilled water, may exist in two forms, as insoluble calcium phosphate and as the mono ester of phosphoric acid—calcium salt with the carbohydrate. In order to render the calcium phosphate soluble and to mobilize the calcium of the ester salt it is necessary to conduct the dialysis (and electrodialysis) at a low pH. Under this condition of low pH there is a marked danger, due to the long time usually required for electrodialysis, that some hydrolysis of the polysaccharide will occur. The following procedure has been found effective in removing any insoluble calcium phosphate (not esterified), together with practically all counter ions (including calcium) combined with the esterified phosphorus, from ungelatinized starch without causing any measurable hydrolysis. The starch sample (granules) is mixed, in a tall cylinder, with two to three times its volume of a 0.1 *N* hydrochloric acid solution saturated with sodium chloride. This is allowed to stand with occasional shaking at room temperature for an hour, after which the granules are allowed to settle and the supernatant solution is removed and replaced by a similar volume of 0.1 *N* acid solution half saturated with sodium chloride which is allowed to remain in contact with the granules for one hour. The procedure is repeated with a solution of the 0.1 *N* acid one-quarter saturated with sodium chloride and finally with repeated changes of distilled water until, after standing for a few minutes, the test for chloride is quite faint in the contact water. In this process the acid reaction serves to mobilize the calcium of the phosphate and ester-phosphate. The sodium chloride, by base exchange action, causes the replacement of calcium ions in the starch granule by sodium, and the exhaustive washing serves to remove most of the excess salt and the acids.

The washed starch granules are then packed into the thin center compartment of an efficient three-compartment electrodialysis apparatus and electrodialyzed. In this apparatus, the middle compartment was only one cm in thickness and a D.C. voltage drop of 100–150 volts per cm could be maintained across this starch diaphragm until most of the diffusible ions were removed. The entire operation may be accomplished in an elapsed time of 12 to 15 hours. During the electrodialysis the temperature of the starch was never allowed to go above 30°C, the dialyzer being kept cool by a glass cooling coil placed in the center compartment. The calcium contents of the electrodialyzed starches given in Table I indicate that an efficient removal of this cation was accomplished, and the alkali lability values on the starches prior to and after the treatment indicate that no detectable hydrolytic degradation occurred.

Analyses of the Starches. The calcium contents of the starches were determined in duplicate by the method of Kramer and Tisdall (1921). Samples of starch varying from 5 to 15 g of dry starch were dried in a platinum dish at 105°C for 12 hours, and the dry weight determined. The dried samples were ashed by placing in a cold muffle and heating slowly to 400°C until charred (frothing was prevented in this manner), then heating at 600°C for 6 hours. The ash was fused with sodium carbonate, dissolved in 6 *N* hydrochloric acid, and the solution transferred to a 50 ml conical centrifuge tube. Five ml of saturated ammonium oxalate and 2 drops of bromcresol purple were added and the pH adjusted with ammonium hydroxide to about pH 6.2. The sample was allowed to stand for 6 hours, centrifuged, washed with 2% ammonium hydroxide, centrifuged and drained, and 10 ml of 3 *N* sulfuric acid added. The sample was then titrated with 0.01 *N* potassium permanganate and the calcium content calculated. Agreement between duplicates was usually within 3%. Average values are given in Table I.

Phosphorus contents of the starches were determined in triplicate by a modification of the colorimetric method of Fiske and Subbarow (1925) in which samples of dry starch weighing 0.1 g to 0.3 g (1.0 g samples in case of the corn starches) were wet-ashed with sulfuric and nitric acids and the color which developed upon the addition of ammonium molybdate and 1-amino-2 naphthol-4-sulfonic acid was estimated in a Coleman spectrophotometer at wave length 725 mμ. Phosphorus content was obtained by comparison with a standard curve made with the same procedure and instrument. The triplicate determinations usually agreed within 10%. Average values are given in Table I.

Alkali lability values on the starches were determined by the method of Schoch (1940).

pH Measurements on the Starch Pastes. One gram (dry weight basis) samples of the electrodialyzed starch granules were placed in 50 ml Erlenmeyer flasks and 30 ml of carbon dioxide free distilled water containing appropriate increments of carbon dioxide free sodium hydroxide were added. Each sample was gelatinized by placing the flask, while being rotated to maintain the starch granules in suspen-

TABLE I

CALCIUM, PHOSPHORUS CONTENTS, ALKALI LABILITY VALUES OF STARCHES BEFORE AND AFTER BASE-EXCHANGE-ELECTRODIALYSIS AND ACIDIC CAPACITIES OF THE ELECTRODIALYZED STARCHES

Starch	Calcium (per g starch)				Phosphorus (per g starch)				Alkali lability		Acidic capacity (per g starch)			
	Before elect.		After elect.		Before elect.		After elect.		Value		Below pH 5		Between pH 5 and pH 8.7	
	mg	meq	mg	meq	mg	meq	mg	meq	Before elect.	After elect.	meq	A[1]	meq	B[1]
Potato PI	0.14	0.007	0.006	0.0003	0.430	0.014	0.413	0.013^8	4.3	5.2	0.012^6	.95	0.021^5	1.61
Potato PII	.11	.005	.014	.0007	.515	.017	.492	$.015^9$	—	—	$.014^4$	.91	$.024^3$	1.53
Potato PIII	.32	.016	.019	.0009	.675	.022	.640	$.020^7$	7.6	6.4	$.019^5$	.94	$.031^2$	1.51
Potato PIV	—	—	.012	.0006	.520	.017	.502	$.016^2$	—	—	$.015^1$	.93	$.024^3$	1.49
Potato PV	—	—	.016	.0008	.510	.016	.415	$.013^4$	—	—	$.012^1$	.90	$.021^5$	1.60
Potato PVI	.49	.024	.011	.0005	.693	.022	.650	$.021^0$	6.1	6.3	$.019^6$	.93	$.027^6$	1.31
Potato PVI DF	—	—	—	—	—	—	.612	$.019^7$	—	—	$.018^6$	.94	$.024^5$	1.24
Potato PVI MH	—	—	—	—	—	—	.460	$.014^9$	—	—	$.013^8$	.94	$.018^2$	1.22
Corn C	.18	.009	.012	.0006	.154	.005	.137	$.004^4$	13.2	12.9	0	—	$.022^0$	5.50
Corn CDF	.13	.006	.001	—	.095	.003	.082	$.002^6$	13.2	12.7	0	—	$.002^4$	0.92
Wheat W	.31	.015	.011	.0005	.570	.018	.550	$.017^8$	—	—	0	—	$.006^0$	3.00
Wheat WDF	.22	.011	.008	.0004	.508	.016	.402	$.013^0$	11.7	10.2	0	—	$.001^6$	0.08

[1] $A = \frac{\text{meq acid titrated below pH 5.0}}{\text{meq phosphorus}}$

[2] $B = \frac{\text{meq acid titrated between pH 5.0 and pH 8.7}}{\text{meq phosphorus}}$

sion, in a boiling water bath for 10 minutes. The pastes so obtained were cooled to room temperature and their pH values determined with a glass electrode assembly employing a L. and N. vacuum tube voltmeter, student potentiometer, and type R galvanometer. With this apparatus, pH values accurate to 0.01 pH units were attained. Titration curves obtained by plotting the observed pH values against the equivalents of sodium hydroxide added to the corresponding samples were smooth and accurately repeatable.

Results and Discussion

Titration Curves for Electrodialyzed Potato Starches. The titration curves for the eight samples of potato starch, three of which are shown in Figure 1, all exhibit the two distinct buffer ranges characteristic of the first and second hydrogens of phosphoric acid. Titration of the first hydrogen is complete at about pH 5.0 and of the second hydrogen at about pH 8.75. The amount of alkali necessary to complete the neutralization of the first hydrogen is consistently from 5 to 10% less than the amount calculated as necessary from the phosphorus content of the electrodialyzed starch (A in Table I). This may be taken to indicate that the base exchange-electrodialysis process failed to remove a small amount of counter-ion. Analysis for calcium shows that not all of the retained counter-ion was calcium and indicates that a small amount of sodium must also have been retained. Dissociation constant calculations for the most acid hydrogen in the potato starches using the Van Slyke (1922) equation, $\mathrm{Ka} = \frac{[\mathrm{H}]([\mathrm{B}] + [\mathrm{H}])}{[\mathrm{S}] - [\mathrm{B}] - [\mathrm{H}]}$, yielded values for pKa of 2.7 for PVI to 3.1 for PI and PV which are considerably higher than that accepted for the first hydrogen of orthophosphoric acid (pKa = 2.03).

In Figure 1 is given the titration curve for an aqueous solution of orthophosphoric acid containing the same concentration of phosphorus as the potato sample PVI titrated. The shift of the titration curve, corresponding to the first hydrogen of the starch phosphoric acid, toward the alkaline range as compared with that of the free phosphoric acid may be due to an actual decrease in the dissociation tendency of this acidic hydrogen when the phosphoric acid is esterified with the starch. However, the dissociation tendencies of both the first and second hydrogens of phosphoric acid, when esterified with glucose, are reported to have increased as a result of the ester formation. The pK_1 value for glucose-1-phosphate has been reported by Cori, Colowick, and Cori (1937) to be 1.11, while that for glucose-6-phosphate was found to be 0.97 by Meyerhof and Lohmann (1927). It seems that a more likely explanation for the decrease in pK_1, observed in this case, is that it is due to a Donnan equilibrium effect. The electrode measures the hydrogen ion activity of the solution outside but in Donnan equilibrium with the region inside the swollen starch granules. The deviation of an observed pK value from its true value would be in the direction observed and would be greatest for starches containing the lowest amounts of phosphorus. No dependable value can, therefore, be assigned for the pK_1 from our titration data, although it is probable that the starch-phosphoric acid is as strong an acid as orthophosphoric acid itself.

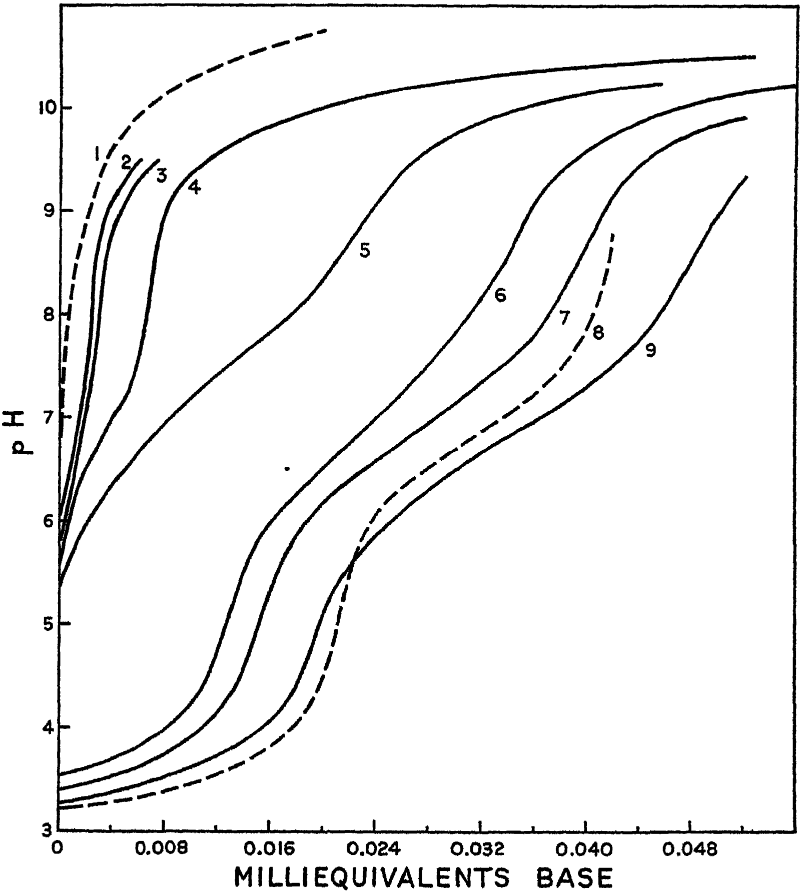


Fig. 1. Titration curves on electrodialyzed starches. Curve 2—Methyl alcohol-extracted wheat starch, WDF; Curve 3—Methyl alcohol-extracted corn starch, CDF; Curve 4—Commercial wheat starch, W; Curve 5—Commercial corn starch, C; Curve 6—Cobbler potato starch, PI; Curve 7—Red Warba potato starch, PII; Curve 9—Commercial potato starch, PVI; Curve 1—H_2O; Curve 8—Orthophosphoric acid solution of same phosphorus content as that of potato starch PVI. Volume of the solutions = 30 ml. Starch solutions contained 1 g each of starch.

The leg of the titration curve lying between pH 5.0 and 8.75, while yielding an over-all value of pK_2 (using the Henderson Hasselbalch equation) which agrees well with the second hydrogen of orthophosphoric acid (observed $pK_2 = 6.75$ for starch PVI, while accepted value for orthophosphoric acid is 6.8), requires more alkali to titrate it than would be expected from the amount of total phosphorus found in the starch (B in Table I). The latter accounts for only 62 to 76% of the alkali needed, this varying with the source of the starch. Obviously some group besides the second hydrogen of starch phosphoric acid ester is reacting with alkali in this pH range. Because of this and the superimposed Donnan effect, an accurate value for pK_2 of the starch-phosphoric acid cannot be given. It may be safely concluded, however, that the first and second hydrogens of potato starch-phosphoric acid ester have dissociation tendencies which are as great as those of the corresponding hydrogens of unesterified orthophosphoric acid.

The phosphorus esterified with potato starch is not constant per gram of starch, but varies with the source of the starch. From the data presented here it seems probable that there is less variation in bound phosphorus between starches of potatoes of the same variety grown on different fertilizer plots (PI and PV) than there is between different varieties grown on the same soil (PI, PII, PIII, PIV). Only small amounts of phosphorus are removed from any of the starches by the base exchange and electrodialysis treatment. Hot methanol extraction followed by the base exchange-electrodialysis treatment removed only a little more of the potato starch phosphorus. Moisture-heat treatment followed by base exchange and electrodialysis removed about a third of the potato starch phosphorus. It is probable that the amount of phosphorus mobilized in all these cases represents a corresponding degree of hydrolysis of the starch-phosphoric acid linkage in the potato starch. The esterified phosphorus content of potato starch can thus be expected to be a function to some extent of the previous treatment of the starch.

Titration Curves for Cereal Starches. The titration curves for corn and wheat starches, both before and after hot methanol extraction, show distinct differences from those of the potato starches. In the first place there is no evidence of a buffer range corresponding to the first hydrogen of phosphoric acid. Wheat starches, before and after methanol extraction, show a small degree of buffer capacity in the pH region in which the second hydrogen of potato starch phosphoric acid ester is titrated, but this is much less than would be expected from the phosphorus contents of these wheat starches and is not greatly changed by the methanol extraction of the starch. Corn starch, undefatted, shows a marked buffer capacity in this region of pH which is considerably greater than could be accounted for by its phosphorus content. This buffer capacity of corn starch is almost completely removed when the starch is defatted. Evans and Briggs (1941) found that the free fatty acid content of this starch was 0.0052 g per gram of starch, these acids having an average molecular weight of 275. This is equal to 0.02 milliequivalents of fatty acid per gram of starch. The difference observed in the buffer capacity of corn starch (in this pH region) before and after defatting was .019 milliequivalents. Obviously the adsorbed but unesterified fatty acids of the corn starch are responsible for most of this buffer capacity of the undefatted corn starch. It is surprising that fatty acids should be titratable in this pH range (pKa = 7.5) since most such acids in aqueous solution act as considerably stronger acids (pKa values approximately 5). The strongly adsorbed state of the free (unesterified) fatty acids in starch apparently causes a shift in their dissociation tendency toward that of a weaker acid.

The very small residual buffer capacity of defatted corn starch may well be due to a residual amount of fatty acid not removed by the methanol extraction. The low buffer capacity of the wheat starches in this same pH region may also be due to small amounts of unesterified but strongly adsorbed fatty acids in the starch. No such adsorbed fatty acids have been found in potato starch, yet we find a considerable buffer capacity for potato starch in this same pH range which cannot be accounted for by the phosphoric acid present. Also, exhaustive methanol extraction of potato starch fails to reduce this extra buffer capacity to any appreciable degree. There must, therefore, remain the possibility that there is some material (or group) present in potato starch, and possibly in wheat and corn starches, which is not removable by methanol extraction or by exhaustive electrodialysis and which shows a buffer capacity in the pH region between 5.0 and 8.7. This might be silicic acid or some protein constituent. No definite identity can be conferred on it at present.

If any of the phosphorus present in the cereal starches were combined as simple ester with the starch, either as mono or di ester, it should be detectable in the titration curve of the electrodialyzed starch as a buffer zone corresponding to the first hydrogen of orthophosphoric acid (at the appropriate dilution corresponding to the amount of phosphorus present in such form in the starch). Even very small amounts should be detectable. The curves for the cereal starches show no indication that any such phosphorus exists in these starches. This does not necessarily mean that none of the phosphoric acid in corn or wheat starch is in ester combination with the starch. It does mean, however, that, if such an ester exists, either it is the tri ester or the phosphoric acid is in mono ester combination with starch and has a strongly alkaline group esterified through a second group. The zwitter ion which would result would act similarly to lecithin where electrodialysis can take the pH of its solution only to the isoelectric point of the zwitter ion The fact that a large part of the phosphorus of wheat starch is of a phospholipid nature could emphasize the probability that, if any part were in combination directly with starch, it too might be similarly combined with a basic group as in the phospholipid.

Above a pH of 9.0 *all* the starches show a very strong buffer action toward added alkali, the extent of which is apparently independent of the source of the starch. In this alkaline region there is either a strong tendency to adsorb alkali, or, more likely, one or more of the hydroxyl groups on the sugar units of the starch molecule evince their acidic nature by reacting as acids to form salts with the added alkali.

Summary

Titration curves on exhaustively electrodialyzed potato starches show buffer ranges which correspond well with those of the first and second hydrogens of orthophosphoric acid. That corresponding to the first hydrogen agrees well with that expected from the total phosphorus content of the starch. That corresponding to the second hydrogen is from one-fourth to one-third greater than can be accounted for by the phosphorus present in the starch.

Titration curves for exhaustively electrodialyzed wheat and corn starches show no buffer capacity in the pH range in which the first hydrogen of the potato starch acid is titratable. Both show buffer capacity in the pH region in which the second hydrogen of potato starch acid is titratable, but their buffer capacities in this region are not related to the phosphorus contents of the starches. With corn starch this buffer capacity is correlated with the unesterified adsorbed fatty acid content of the starch. It is postulated that the slight buffer capacity of wheat starch in this pH region may be due to the presence of free fatty acids or to groups (or material) similar in nature to the unidentified material in potato starch which is responsible for the excess buffer capacity of this starch over that accountable for on the basis of its phosphorus content.

None (or no detectable amount) of the phosphorus present in cereal starches exists in the mono-orthophosphoric acid ester form which is characteristic of all the phosphorus of electrodialyzed potato starch.

Above pH 9.0 all starches show an equal tendency to neutralize alkali, and this occurs to a relatively much greater degree than is characteristic of the buffer capacity of any starch at lower pH ranges. This probably involves the ionization of hydrogen in the hydroxyl groups of the sugar monomers of the starch.

Literature Cited

Cori, C. F., Colowick, S. P., and Cori, G. T.
1937 The isolation and synthesis of glucose-1-phosphoric acid. J. Biol. Chem. 121: 465–477.
Evans, J. W., and Briggs, D. R.
1941 The lipids of corn starch. Cereal Chem. 18: 443–461.
Fiske, C. H., and Subbarow, Y.
1925 The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375–400.
Kramer, B., and Tisdall, F. F.
1921 A simple technique for the determination of calcium and magnesium in small amounts of serum. J. Biol. Chem. 47: 475–481.
Lehrman, L.
1945 Extraction of fat and phosphorus from wheat starch. J. Am. Chem. Soc. 67: 1541–1542.
Meyerhof, O., and Lohmann, K.
1927 Ueber die enzymatische Milchsaeurebildung in Muskelextract. Biochem. Z. 185: 113-164.
Posternak, T.
1935 Sur le phosphore des amidons. Helv. Chim. Acta 18: 1351–1369.
Sair, L., and Fetzer, W. R.
1944 Water sorption by starches. Ind. Eng. Chem. 36: 205–208.
Samec, M.
1927 Kolloidchemie der Starke. T. Steinkopff, Dresden and Leipzig.
—— and Klemen, R.
1931 Studies on plant colloids. XXVIII. Properties of different types of starch. Kolloidchem. Beihefte 33: 254–268.
Schoch, T. J.
1942 Non-carbohydrate substances in the cereal starches. J. Am. Chem. Soc. 64: 2954–2956.
—— and Jensen, C. C.
1940 A simplified alkali-lability determination for starch products. Ind. Eng. Chem. (Anal. Ed.) 12: 531–532.
Van Slyke, D. D.
1922 On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer solution. J. Biol. Chem. 52: 525-570.

PREPARATION AND NITROGEN CONTENT OF SOYBEAN PROTEIN

WILLIAM G. SMILEY and ALLAN K. SMITH

Northern Regional Research Laboratory,[1] Peoria, Illinois

(Received for publication November 21, 1945)

The term "soybean protein" usually refers to the curd which is precipitated from an aqueous or alkaline extract of soybean meal by addition of acid. It has become a commercial product of considerable importance. The solution remaining after removal of the curd is known as whey and has no value at present, although it is well known to contain additional protein. Since water alone extracts at least 90% of the nitrogenous material from soybean meal and alkali practically all of the nitrogen, the curd and whey contain nearly all the protein of the soybean, although such high yields are not attained in commercial operation.

The separation of soybean proteins was first studied by Meissl and Böcker (1883), and later more extensively by Osborne and Campbell (1898) who named the principal protein "glycinin." Osborne's glycinin is defined as "the salt solution soluble globulin which separates after dialysis"; however, it has not been identified as a single protein. Thus, the question is raised as to the purity of the "soybean protein" prepared by commercial methods in comparison with Osborne's "glycinin."

In the absence of crystallinity or distinctive physical properties, the nitrogen content is the most convenient method of determining the purity of a protein. The variations in nitrogen content of different preparations of soybean protein from the same source material might be due to nonprotein impurities or to fractionation into proteins of different composition. We shall present evidence that the former appears to be the more important factor. Jones and Csonka (1932) and also Ryndin, Morozov, and Salchinkin (1936) separated glycinin into fractions having different physical properties, while Csonka and Jones (1933) showed that the nitrogen content and amino acid composition of glycinin vary with the variety of soybean.

The following methods have been used in separating soybean protein and glycinin from the nonprotein parts of soybean meal and from the minor proteins (albumin and proteose):

A. Extraction with water and precipitation with acid.
B. Extraction with dilute alkali and precipitation with acid.

---

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

C. Extraction with a salt solution (usually 1–2N NaCl) and precipitation by dialysis or dilution.

D. Extraction with water or salt solution, precipitation by saturating with ammonium sulfate, redispersing in water, and precipitation by dialysis.

Methods B and C may be repeated one or more times to obtain a purer product. Methods A and B are essentially the procedures used industrially; most chemists have followed Osborne and Campbell in preferring C and D. Method B gives the largest yield, since it includes the less soluble part of the protein. The insoluble protein fraction in the meal residue can be decreased by fine grinding of the meal (Smith, Circle, and Brother, 1938). It is possible that it does not actually represent a distinct protein, but protein retained in intact cells as suggested by Woodruff, Chambers, and Klaas (1938).

Table I shows the nitrogen content of various protein preparations reported in the literature. The methods of preparation are indicated by the letters used above, successive letters showing purification steps. An asterisk indicates that the final precipitate was dehydrated with alcohol.

TABLE I

NITROGEN CONTENT OF GLYCININ AND SOYBEAN PROTEIN FROM THE LITERATURE

Authors	Variety of bean	Method of preparation	N[1]	Yield[2]
			%	%
Osborne and Campbell (1898)	"Yellow soybean"	D *	17.14	7.7[3]
		DCC *	17.72	
	"Kiyusuki diadzu"	D *	17.45	6.1[3]
		DCC *	17.48	
Jones and Moeller (1928)	"Yellow soybean"	"Method of Osborne and Campbell"	17.04	—
Csonka and Jones (1933)	Illini	C[4]	17.74	—
	Dunfield	C[4]	17.37	—
	Manchu	C *	17.28	—
	Peking	C *	17.19	—
	Dixie	C *	16.60	—
Hartman and Cheng (1936)	—	CC[4]	17.61	10.0
Ryndin (1936)	—	CC *	17.35	5.4
Woodruff, Chambers, and Klaas (1938)	Illini	A *	16.40	25.3
	(commercial flakes)	C *	16.44	10.4
Smith and Circle (1939)[5]	Illini	A	15.68	29.4
Pucher and Nolan; see Vickery (1945)	Illini	CC[1]	16.93	—

[1] On ash-free dry basis.
[2] On weight of oil-free meal.
[3] Total of several fractions.
[4] Dried with acetone.
[5] Not specially prepared for high purity.
* Indicates dehydration by alcohol.

A. Water extraction-acid precipitation.
C. Salt extraction-precipitation by dialysis.
D. Water or salt extraction-ammonium sulfate precipitation.

The range of variation in the nitrogen content seems quite surprising. Jones (1931) showed that the nitrogen content of gliadin and glutenin prepared from various varieties of wheat by many different workers was fairly constant, the maximum variation being 0.68%, and asserted that the same was true of other vegetable proteins. The data on glycinin and soybean curd protein in Table I differ more widely than this, even if we confine ourselves to one paper (Csonka and Jones) or to one variety (Illini).

The nitrogen content of soybean protein is of importance also because of the use of factors in food and nutrition studies to determine the protein content of soybean products. The conventional factor of 6.25 is still in use for this purpose. Jones (1931), while pointing out the fundamental weaknesses of this method of determining protein, nevertheless recommended a factor based on the nitrogen content of glycinin, taken as 17.5%. There appears to be little value in adopting a new factor if the nitrogen content of glycinin is as uncertain as Table I indicates.

In addition to comparing the purity of soybean protein prepared by commercial procedures with Osborne's glycinin, the object of the present work was to determine the effect of various factors on the nitrogen content of soybean protein, and especially the relative importance of the source of material, and of the method of preparation in accounting for the variations. We have found that the nitrogen contents obtained by different methods of purification tend to reach an upper limit that is not very different for different varieties and crop years. Most of the variation in our preparations appears to be due to a nonprotein impurity.

Methods and Materials

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, using a mercury catalyst and a one-hour digestion. Chibnall, Rees, and Williams (1943) have shown that a longer period of digestion is essential for egg albumin, and raised the question as to the accuracy of nitrogen determination on other proteins when a short digestion period is used. To check the feasibility of the method for isolated soybean protein, a series of tests was run using 1-, 3-, and 7-hour digestion periods with mercury and copper sulfate catalysts. The results are shown in Table II, where each value is the average of two or more determinations.

From these results, and a Dumas nitrogen determination on the same sample which gave a value of 15.83%, it was concluded that, for an isolated soybean protein, a digestion period of one hour was sufficient. Ash was determined in platinum crucibles at 650°C. Por-

TABLE II

THE EFFECT OF DIGESTION TIME ON THE KJELDAHL NITROGEN DETERMINATION FOR ISOLATED SOYBEAN PROTEIN

Date	Catalyst	Digestion period and percent nitrogen		
		1 hour	*3 hour*	*7 hour*
2-2-44	Hg	15.71	—	—
6-8-44	Hg	15.62	—	—
6-27-45	Hg	—	15.74	15.78
7-2-45	Hg	15.87	—	15.80
6-27-45	$CuSO_4$	—	15.75	—
7-2-45	$CuSO_4$	—	—	15.83
Average	—	15.73	15.75	15.80
Dumas nitrogen	—	15.83	—	—

celain crucibles gave irregular and much higher results. Moisture was determined by drying in a vacuum oven at 105°C for 5 hours. All determinations were made at about equilibrium moisture content, and the nitrogen values corrected for ash and moisture. This is preferable to attempting to analyze the very hygroscopic dried protein.

With the exception of Csonka and Jones (1933), who used ethyl ether, all the authors quoted in Table I used light hydrocarbons (petroleum ether, benzine) to extract the oil from their soybean meal. It has been found in this laboratory (Beckel and Smith, 1944; Belter, Beckel, and Smith, 1944) that soybean meal from which the oil has been extracted with ethanol gives a much lighter-colored protein than meal extracted with other solvents, suggesting that some impurity has been removed. In this study, soybean protein has been prepared from meals extracted with ethanol and with a commercial petroleum ether (Skellysolve F), and the nitrogen contents have been compared. The varieties and crop years used were: Illini, 1941, 1942, 1943; Peking, 1941; Mandarin, 1942; Lincoln, 1943; and Willomi (81044), 1943.

After cracking, dehulling, and flaking, the beans were extracted in a modified Soxhlet-type extractor in which the solvent entered the extractor through a condenser; thus the flakes were kept substantially at room temperature. Soybean protein was also prepared from flakes which were ethanol-extracted on a pilot-plant scale at about 60°C, and from commercial solvent-extracted "brew" flakes having a high water-soluble protein content. For laboratory protein preparations, the flakes were ground in a hammer mill. Results are also given on some proteins prepared in a pilot plant (Belter, Beckel, and Smith, 1944).

Protein Preparations. Table III shows the methods of preparation and nitrogen contents of the protein prepared in this laboratory. The

TABLE III

Laboratory Soybean Protein Preparations

No.	Variety and year	Solvent and method	N[1]	Yield[2]
			%	%
1	Illini 1941	Pet. ether AC	16.73	-
2	Illini 1942	Pet. ether C	16.37	—
3	Illini 1942	Pet. ether CC	16.68	23
4	Illini 1942	Pet. ether CCC	16.51	-
5	Illini 1942	Pet. ether D	16.48	19
6	Illini 1942	Pet. ether AB	15.91	--
7	Illini 1942	Pet. ether ABBB	15.98	26
8	Illini 1942	Pet. ether ABBBBB	15.99	—
9	Illini 1942[3]	Pet. ether A pH 5.4	16.18	32
10	Filtrate from No. 9	pH 4.0	14.95	5
11	Illini 1942	Ethanol B[4]	16.16	--
12	Illini 1942	Ethanol BB	16.27	37
13	Illini 1942	Ethanol BBB	16.32	--
13a	Illini 1942	Ethanol CC	16.67	Low
14	Illini 1943	Ethanol CC *	16.62	--
15	Illini 1943	Ethanol A *	16.47	--
16	Illini 1943	Ethanol ABB *	16.36	—
17	Illini 1943	Ethanol B *	16.00	34
18	Illini 1943	Ethanol BBB[4]	16.13	—
19	Peking 1941[3]	Pet. ether AB	15.30	39
20	Peking 1941[3]	Pet. ether ABB	15.31	--
21	Peking 1941	Pet. ether A	15.58	-
22	Peking 1941	Pet. ether ABB	15.78	36
23	Peking 1941	Ethanol A	16.08	—
24	Peking 1941	Ethanol ABB	16.48	32
25	Lincoln 1943	Ethanol B	15.41	—
26	Lincoln 1943	Ethanol BBB	16.26	38
27	Lincoln 1943	Ethanol BBBB	16.20	—
28	Willomi 1943	Ethanol AB	16.78	—
29	Willomi 1943	Ethanol ABB	16.90	24
30	Willomi 1943	Ethanol B	15.43	—
31	Willomi 1943	Ethanol BB	15.83	43
32	Willomi 1943	Ethanol BBB	16.19	—
33	Mandarin 1942	Pet. ether A	15.86	—
34	Mandarin 1942	Pet. ether A *	16.17	--
35	Mandarin 1942	Pet. ether ABB	16.05	—
36	Mandarin 1942	Pet. ether ABB *	16.32	--
37	Mandarin 1942	Ethanol A *	16.59	20
38	Mandarin 1942	Ethanol BB	16.70	--
39	Mandarin 1942	Ethanol BBB	16.88	38
40	Mandarin 1942	Not extracted AB *	16.21	--

[1] On ash-free dry basis.
[2] Grams of air-dry protein per 100 g of air-dry, fat-free meal.
[3] Including hulls—all others dehulled.
[4] Extracted in pilot plant at 60°C.
* Indicates dehydration by alcohol.

A. Water extraction—acid precipitation.
B. Alkali extraction—acid precipitation.
C. Salt extraction—precipitation by dialysis.
D. Water extraction—ammonium sulfate precipitation.

methods of preparation are designated by the same symbols as in Table I. In Method B, 0.1% sodium hydroxide was usually used in the original extraction, while in the reprecipitation steps, only enough alkali was used to disperse the protein (pH, about 9). Precipitation in Methods A and B was at pH 4.1–4.5, usually with sulfuric acid. In Method C, 6% (approximately 1N) sodium chloride was the dispersing agent; following dialysis, the pH was adjusted when necessary to the above range. In succeeding steps, dilution with several volumes of water was used in place of dialysis. Method D was carried out by water extraction (Osborne and Campbell used salt extraction). Dehydration by alcohol is indicated by an asterisk. In other cases, the glycinin was dried at 65°C in a forced-draft oven. Yields are given only where at least two extractions were made on the meal, in order to obtain maximum yield.

The separation of the first extract from the insoluble residue has always been a difficulty in both laboratory and large-scale production of protein. In these experiments, this was done by centrifuging at about 2000 *g*. Especially with water extraction, the boundary between the extract and the residue is not sharp. This is a possible cause of irregularities in the nitrogen content. Sample 13A, Table III, was given a special filtration treatment with macerated filter paper. After the first salt dispersion, it was filtered twice, and after the second dispersion, it was filtered three times.

The pilot-plant protein preparations are given in Table IV. Unground flakes were used, "commercial" and "ethanol" referring to commercial hydrocarbon-extracted flakes, and pilot-plant ethanol-extracted flakes, respectively. Tap water was used for the alkaline extractions, and distilled water for No. 41. Sulfuric acid was the precipitating agent, except for No. 46 which was precipitated with sulfur dioxide. Sodium bisulfite was used for its bleaching and preservative action.

Discussion

Using the highest nitrogen content obtained for each variety in Table III, we find a variation from 16.26% (Lincoln) to 16.90% (Willomi). This range is no larger than that found for wheat proteins (Jones, 1931), and is in contrast to the large variations shown in Table I. We are unable to explain why no glycinin prepared in this laboratory has ever given as high values of nitrogen as those of Osborne and Campbell (1898), Csonka and Jones (1933), or Hartman and Cheng (1936). However, values in the range of 16.90 to 17.00% nitrogen for samples of protein prepared by very careful work of Dr. G. W. Pucher and Mr. L. S. Nolan in H. B. Vickery's laboratory (Vickery, 1945) are consistent with our results.

More important than variations due to variety and crop year are the variations caused by the method of preparation. These variations are not due to fractionation of the protein, but rather to varying degrees of removal of a nonprotein impurity. This conclusion is based on the following facts:

(1) Some of the preparations of highest nitrogen content which were made from ethanol-extracted meal, as Nos. 11-13, 23–24, and

38–39, were obtained in sufficiently large yields to indicate that they include practically all of the protein. This appears to be the most effective way of removing the impurity.

(2) Dehydrating the wet curd with ethanol also raises the nitrogen content, although not to the same level as ethanol extraction of the meal (compare Nos. 33 37).

(3) Extracting the dried protein with hot ethanol in a Soxhlet extractor raised the nitrogen content in the case of a pilot-plant preparation from commercial flakes (Nos. 41–42), but did not do so for a similar preparation from ethanol-extracted flakes (Nos. 44–45). In the former case, the ethanol extract from 100 g of protein yielded about 4 g of a brown syruplike material; in the latter, less than 0.1 g was obtained.

(4) Methods C and D, which involve a reduced yield and hence considerable fractionation, do not give a much higher nitrogen content than ethanol extraction followed by Method A or B (compare Nos. 2–5, 11–13, 14–18).

The nature of the impurity is still in doubt; it appears to be a complex mixture. The "syrup" mentioned above contains 1.30% nitrogen and 0.79% phosphorus. Acid hydrolysis of the syrup yielded phosphoric acid, fatty acids, and choline (precipitated as the reineckate), indicating the presence of lecithin. Carbohydrates are also present (Molisch reaction). The syrup is largely soluble in most organic solvents and partly so in water. Phosphatides probably act as dispersing agents. The purifying effect of Method C is in harmony with the fact that salts precipitate dispersed phosphatides.

Most of our preparations were made from dehulled beans. Preparations 19–22 show that the inclusion of the hulls adds a further impurity to the protein, as is also shown by its darker color. Soybean hulls contain about 10% of water-soluble material, and the water extract is dark brown.

Preparations 9 and 10 show another method of obtaining a purified protein. A water extract of a petroleum ether-extracted meal, including the hulls, was precipitated at pH 5.4 to give protein having a nitrogen content of 16.18%. The "whey" from this precipitate was adjusted to pH 4 and gave a further small precipitate with a nitrogen content of only 14.95. Evidently, the bulk of the impurity is precipitated at the lower pH. This method offers some difficulty in practice because of the poor coagulation and settling of the precipitate, but it has the advantage of being applicable to present commercial soybean flakes.

Methods C and D (preparations 2 and 5) are about equal to alcohol extraction followed by Method A (preparation 15) in purifying effect, but the latter is more practical for large-scale production. More than two precipitations are of little or no advantage whether Method B or C is used. Method A would be expected to be better than B for the first extraction, but this is not always the case; Method B not only gives the larger yield but sometimes the higher nitrogen content as well.

The four pilot-plant preparations shown in Table IV illustrate the variations in nitrogen content of glycinin which is encountered in large-scale production. The use of flakes rather than ground meal, washing by settling and decantation, and the considerable time required may be factors in this variation. It is rather surprising that a pilot-plant protein, No. 46, has the highest nitrogen content (17.02%) we have encountered. Preparation 47 represents an attempt to duplicate pilot-plant conditions in the laboratory, including the use of unground hot ethanol-extracted flakes and the addition of sodium sulfite. The result agrees with preparation 44 rather than 46.

TABLE IV

PILOT PLANT-SOYBEAN PROTEIN PREPARATIONS

No.	Flakes	Dispersing agent	N[1]
			%
41	Commercial	H_2O	15.28
42	(No. 41 after ethanol extraction)		15.70
43	Commercial	NaOH	16.32
44	Ethanol	$NaOH+Na_2SO_3$	16.50
45	(No. 44 after ethanol extraction)		16.38
46	Ethanol[2]	$NaOH+Na_2SO_3$	17.02
47	Ethanol[3]	$NaOH+Na_2SO_3$	16.65

[1] On ash-free dry basis. [2] Illini soybeans—1942 crop. [3] Prepared in laboratory—see text.

Preparation 40 was made by the unusual method of extracting protein from unextracted (full-fat) soybean flour and removing the oil later. In this process, it is found that the oil disperses and precipitates with the protein, forming a milklike emulsion and showing little or no tendency to separate, even on reprecipitation. By dehydrating the wet curd with ethanol, nearly all of the oil was removed, but the protein was further purified by washing with petroleum ether and extracting with ethanol in a Soxhlet extractor. The nitrogen content compares favorably with other preparations, but in view of the difficulty in the removal of the oil and the ethanol, this method probably is not practical.

Summary

Soybean protein has been prepared by various methods from several varieties of soybeans, and the nitrogen content has been determined. The variations between varieties are small compared to those due to method of preparation, the highest value obtained for each variety ranging from 16.26% (Lincoln) to 16.90% (Willomi). In agreement with other recent workers, we have not been able to obtain a value as high as the usually accepted 17.5%.

The variation in nitrogen content of glycinin preparations from a single source appears to be due to an impurity rather than to fractionation. This impurity is best eliminated by using ethanol as the solvent in extracting the oil.

Acknowledgment

The analyses were made by the Analytical and Physical Chemical Division of this laboratory. The pilot-plant proteins were furnished by Mr. Paul A. Belter.

Literature Cited

Beckel, A. C., and Smith, A. K.
1944 Alcohol extraction improves soya flour flavor and color. Food Industries **16**: 616, 664.
Belter, P. A., Beckel, A. C., and Smith, A. K.
1944 Soybean protein production. Comparison of the use of alcohol-extracted with petroleum-ether-extracted flakes in a pilot plant. Ind. Eng. Chem. **36**: 799–803.
Chibnall, A. C., Rees, M. W., and Williams, E. F.
1943 The total nitrogen content of egg albumin and other proteins. Biochem. J. **37**: 354–359.
Csonka, F. A., and Jones, D. B.
1933 Differences in the amino acid content of the chief protein (glycinin) from seeds of several varieties of soybean. J. Agr. Research **46**: 51–55.
Hartman, R. J., and Cheng, L. T.
1936 Studies on soy-bean proteins. II. An improved method for the preparation of glycinin. J. Chinese Chem. Soc. **4**: 152–156.
Jones, D. B.
1931 Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U. S. Dept. Agr. Circ. 183.
—— and Csonka, F. A.
1932 Precipitation of soybean proteins at various concentrations of ammonium sulfate. Proc. Am. Soc. Biol. Chem. **26**: XXIX–XXX.
—— and Moeller, O.
1928 Some recent determinations of aspartic and glutamic acids in various proteins. J. Biol. Chem. **79**: 429–441.
Meissl, E.; and Böcker, F.
1883 Über die Bestandtheile der Bohnen von Soja Hispida. Sitzber Akad. Wiss. Wien, Math-naturw. Klasse **87**: 372-391.
Osborne, T. B., and Campbell, G. F.
1898 Proteids of the soybean. J. Am. Chem. Soc. **20**: 419 428.
Ryndin, T. V.
1936 Colloid chemical characterization of soybean proteins. Colloid J. (U.S.S.R.) **2**: 811–819.
—— Morozov, A. A., and Salchinkin, A. P.
1936 Physical chemistry of plant proteins. Colloid J. (U.S.S.R.) **2**: 831-839.
Smith, A. K., and Circle, S. J.
1939 Soybean protein. Precipitation from water and alkaline dispersions by acids and by electrodialysis. Ind. Eng. Chem. **31**: 1284–1288.
——, ——, and Brother, G. H.
1938 Peptization of soybean proteins. The effect of neutral salts on the quantity, of nitrogenous constituents extracted from oil-free meal. J. Am. Chem. Soc. **60**: 1316–1320.
Vickery, H. B.
1945 The proteins of plants. Physiol. Revs. **25**: 347–376.
Woodruff, S., Chambers, E., and Klaas, H.
1938 A study of protein extract from soybeans with reference to its use in food. J. Agr. Research **57**: 737–746.

STALING STUDIES ON BREADS CONTAINING WAXY MAIZE STARCH[1]

P. P. Noznick, P. P. Merritt, and W. F. Geddes

Division of Agricultural Biochemistry, University Farm, St. Paul, Minnesota

(Received for publication August 9, 1945)

Researches on the complex phenomena which occur during the staling of bread have associated the decrease in compressibility of the crumb with changes in the starch. The hardening of the crumb is accompanied by a decrease in crumb translucency, in the swelling power in water, in the viscosity of its aqueous dispersions, and by a reduction in the amount of water-extractable polysaccharides. The literature on this subject has been reviewed by Katz (1928), Alsberg (1936), Cathcart (1940), and Radley (1943). As a result of his extensive investigations Katz attributed the staling of bread crumb to a "retrogradation" of the starch, a term which has been loosely used in the literature to denote colloidal changes which occur in starch pastes on standing whereby the starch becomes less soluble. His X-ray studies (Katz, 1930, 1934) indicated that the insolubilization of the starch in a paste involves a transition from the amorphous to the crystalline state and that a similar change occurs when bread stales. Alsberg (1936) has pointed out that the retrogradation theory is not in accord with either the rapidity or the reversibility of the staling process. Even concentrated starch pastes retrograde slowly as compared with the rate at which bread stales, and the retrograded starch is extremely resistant to solubilization.

During the past decade our knowledge of the structure and properties of starch has greatly increased. Although earlier workers had shown that the common cereal and tuber starches could be separated by various means into two fractions which differed in their properties, methods have recently been perfected that provide a clear-cut separation into a straight chain component and a branched chain component, designated as amylose and amylopectin, respectively, by Meyer (1942). The linear component, which can be selectively precipitated from autoclaved starch solutions with butyl or amyl alcohol (Schoch, 1942), with higher fatty acids (Schoch and Williams, 1944), or with certain nitroparaffins (Whistler and Hilbert, 1945), comprises 25 to 30% of the normal starches and forms highly unstable solutions which retrograde rapidly to insoluble aggregates or irreversible gels. The retrogradation of amylose can readily occur because of the ease with which the linear molecules can orient to form

---

[1] Paper No. 2250, Scientific Journal Series, Minnesota Agricultural Experiment Station.

"crystallites." On the other hand, the amylopectin, or branched chain component, forms relatively colloidally stable solutions which exhibit little tendency to retrograde. The starches prepared from the so-called waxy or glutinous varieties of cereals are unique in their properties. Pastes prepared from these "waxy" starches have high hot viscosities, low gel rigidity, and show a remarkably low tendency to retrograde (Brimhall and Hixon, 1939; Hixon and Sprague, 1942; Schopmeyer, Felton, and Ford, 1943). That waxy maize starch is composed almost entirely of the branched chain component, amylopectin, is evidenced by its low alkali number, its failure to give any precipitate with butanol (Schoch, 1942), its low iodine adsorption (Bates, French, and Rundle, 1943), and its osmotic and viscosity behavior (Foster and Hixon, 1943). The absence of any significant amount of the linear component in waxy maize starch, according to Hixon and Sprague (1942), is a possible explanation for its inability to retrograde at all rapidly since the branched molecules "would have difficulty in orienting into insoluble groups of parallel chains, which is characteristic of retrogradation."

Interpreting Katz' retrogradation theory in the light of this newer knowledge of starch chemistry, it may be postulated that bread staling is due to the amylose component of starch. If this assumption is valid, as pointed out by Hixon (1943), bread made with waxy starch should exhibit little tendency to stale. Although waxy or glutinous varieties of corn, rice, millet, and grain sorghum are known, no waxy varieties of wheat have yet been reported. However, the effect of waxy starch on bread staling can be investigated by conducting staling tests on bread made from gluten-starch mixtures. Aitken and Geddes (1938, 1939), Harris (1940), and Aitken and Anderson (1943) have shown that the baking characteristics of flours may be enhanced by the addition of crude gluten which has been dried in a stream of air at 32°C. Sandstedt, Jolitz, and Blish (1939) found that wet gluten and starch may be recombined to form a dough with baking characteristics similar to those of doughs prepared from the original flours. Harris and Sibbitt (1942) and Finney (1943) have also used the synthetic approach in studying differences in the baking value of gluten and starch prepared from different wheat varieties.

In this paper, the results of staling studies on breads made from mixtures of gluten with wheat and waxy maize starches are reported.

Materials and Methods

Preparation of Gluten and Starches. Dried gluten and wheat starch prepared from an unbleached straight grade flour milled from hard red spring wheat in connection with a previous investigation were available for this study. The gluten was prepared by washing the starch from doughs at approximately 0°C. Part of the gluten was frozen at − 16°C, ground, and dried cryoscopically; the part used in this study was dried at room temperature (approximately 25°C) in a stream of air from an electric fan. The starch was removed from the wash water by centrifuging and divided into the upper and lower starch fractions which were frozen and dried cryoscopically. The lower starch fraction was used in preparing the synthetic flours.

Waxy maize and waxy sorghum starches prepared without the use of sulfur dioxide were supplied by the Northern Regional Laboratory, Peoria, Illinois.

Preparation of Synthetic Flours. Synthetic flours containing 12% protein (N × 5.7; 14% moisture basis) were prepared by mixing the appropriate quantities of finely pulverized dried gluten and starch. A series of flours comprising mixtures of 0, 10, 50, and 100% of waxy maize and wheat starches was prepared. In addition, mixtures of waxy sorghum and wheat starches were employed in preliminary studies.

Baking Procedure. The synthetic flours were baked by a micro-baking technique similar to that described by Geddes and Aitken (1935). The regular A. A. C. C. basic formula (*Cereal Laboratory Methods*, 4th ed., 1941) using 6% of sucrose was employed and sufficient ingredients to yield three micro loaves were mixed in a Hobart-Swanson mixer for two minutes. Upon removal from the mixer, three 50-g doughs were scaled, fermented for two hours at 30°C, molded, and proofed in low-form micro pans for 55 minutes at 30°C. The doughs were baked for 20 minutes at 230°C. The three loaves were weighed together, their combined volume determined by seed displacement, and the specific volume computed. Thirty minutes after removal from the oven, the loaves were coated with paraffin and stored in airtight tins at 26°C.

Determination of Staling. Two series of staling studies were made. In the first, the staling of the micro loaves was followed by means of the compressibility technique, as outlined by Platt and Powers (1940); in the second, the crumb swelling technique, as modified by Cathcart and Luber (1939), was used.

Compression measurements were made with the Baker compressimeter in which the usual plunger was replaced by a much smaller one, measuring 3/4 inch in diameter, thus allowing for the testing of the small slices obtained from the micro loaves. Three slices, 0.5 inch thick, were cut from a single micro loaf with the aid of a mitre box, both ends being discarded. Two separate compressions were measured on each slice, one covering the upper, the other the lower half of the slice During each individual determination, the depth of compression or the strain, as measured in mm, was recorded at stresses of 20 and 40 g Tests were made on the three loaves baked from a single mix at intervals of 3, 9, and 27 hours, respectively, after baking, and were duplicated on another series of loaves baked on a different day.

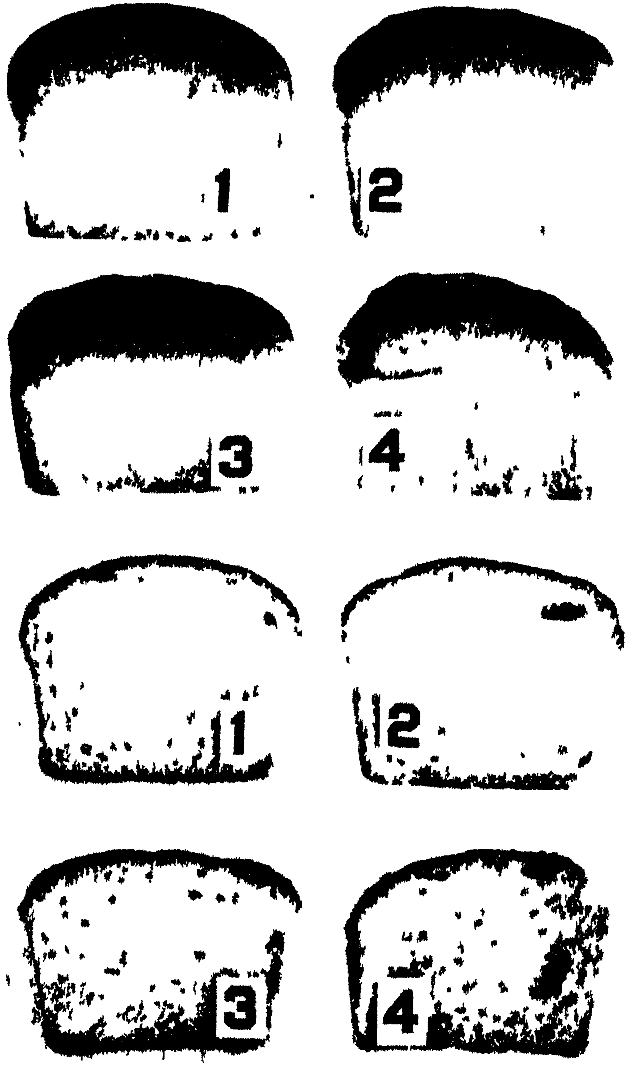


Fig. 1 External and internal views of micro loaves baked from gluten-starch mixtures. In loaf No. 1 wheat starch was used, in loaves No. 2 to 4 the starch component comprised mixtures of wheat and waxy maize starch and contained 10, 50, and 100% waxy maize respectively

The crumb swelling tests were made at 3 and 27 hours after baking and were duplicated on different days.

Results

Increasing the percentage of waxy maize starch in the gluten-starch mixtures increased the absorption and decreased the rate of dough development during mixing; also during fermentation the doughs tended to become dry. As can be seen from Figure 1, increasing the percentage of waxy maize starch produced a decrease in the quality of the bread. The crust became glazed and sugary in appearance and the loaf volume decreased. The crumb became less rigid, resulting in excessive shrinkage upon cooling, and was gummy in nature. These gummy characteristics were so pronounced that it was impossible to slice the bread made from gluten and all waxy maize starch, and compressibility tests could not be made.

TABLE I

EFFECT OF TIME ON COMPRESSIBILITY OF BREAD CRUMB OF LOAVES CONTAINING VARYING AMOUNTS OF WAXY MAIZE STARCH

Composition of starch mixture		Specific loaf volume	Mean compression in mm					
			Stress—20 g Elapsed time—hours			Stress—40 g Elapsed time—hours		
			3	9	27	3	9	27
Wheat %	*Waxy maize* %							
100	0	1.39	0.891	0.52	0.28	1.95	1.02	0.47
90	10	1.43	1.18	0.60	0.35	2.24	1.21	0.59
50	50	1.14	1.21	0.74	0.31	2.18	1.25	0.54

The compressibility tests recorded in Table I show that while waxy maize starch increases the compressibility of the crumb, it does not delay the rate of staling, as measured by the relative changes in compressibility with time of storage. For example, the decreases in crumb compressibility in 24 hours with a stress of 20 g were 0.61, 0.83, and 0.90 mm for 100% wheat starch, 10% waxy maize, and 50% waxy maize starch respectively. The corresponding compressibility decreases for 40 g stress were 1.48, 1.65, and 1.64 mm. Relatively large errors are involved in compressibility measurements. Noznick and Geddes (1943), working with cakes, showed that increased precision is obtained by making the tests at the higher stresses. On this basis, the results obtained at a stress of 40 g are probably the more significant, and it may be concluded that waxy maize starch has relatively little influence on the rate of hardening of bread crumb.

The low gel rigidity of waxy maize starch pastes naturally raises the question as to whether this component of the gluten-starch mixtures contributed materially to the structural rigidity of the crumb. There was an apparent slight increase in crumb compressibility of the bread containing waxy maize starch, but the increase was not progressive as the proportion of this starch was increased. As the applicability of this method of measuring staling in the instance of the gummy crumb obtained when waxy maize starch was present was open to some question, swelling tests were made as an additional measure of staling.

TABLE II

Effect of Time on the Swelling Capacity of Bread Crumb of Loaves Containing Varying Amounts of Waxy Maize

Composition of starch mixture		Specific loaf volume	Volume of centrifugate[1]		Difference in volume
			Elapsed time—hours		
			3	27	
Wheat %	*Waxy maize* %	*cc*	*ml*	*ml*	*ml*
100	0	1.13	4.6	4.1	0.5
90	10	1.20	4.4	4.1	0.3
50	50	0.95	6.0	3.8	2.2
0	100	0.92	7.2	5.4	1.8

[1] Volume for 2 g of crumb (original moisture basis).

The results, recorded in Table II, show that waxy maize starch increases the swelling capacity of the crumb when compared at the same time interval after baking. The presence of this starch resulted in a greater decrease in swelling capacity over the 24-hour time interval than occurred with wheat starch. It is noteworthy, however, that the actual swelling capacity of the crumb in which the starch component consisted entirely of waxy maize starch was somewhat higher after 27 hours' storage than the crumb for all wheat starch after 3 hours' staling. The washings obtained from the crumb became progressively more slimy as the percentage of waxy maize starch was increased.

Discussion

Though limited in scope, these studies show quite definitely that waxy maize starch has a very detrimental effect on dough and bread quality and is of no value in decreasing the rate of staling. In fact, as measured by the change in crumb swelling capacity, the staling rate is increased. Though less complete, additional studies employing waxy sorghum starch gave similar results to those obtained with waxy maize starch.

As waxy starches are essentially free of amylose, the results indicate that the staling of bread at least cannot be solely attributed to the marked retrogradation tendencies of this component of wheat starch. The ease with which stale bread can be freshened, such as by heating at a temperature of approximately 60°C for a few minutes, is not in line with the postulate that the amylose fraction is the primary factor, since retrograded amylose must be subjected to prolonged autoclaving to bring it into solution.

Since the swelling capacity of the crumb in which the starch component consisted only of waxy maize starch markedly decreased with age, it would appear that changes in the amylopectin component of wheat starch may well be a primary factor in the bread staling process if it is assumed that the properties of the amylopectin from these two sources are similar. Such a view is difficult to reconcile with published researches showing that amylopectin solutions exhibit little tendency to retrograde. Yet, it is quite conceivable that retrogradation of the branched chain component of wheat starch may occur under the conditions existing in bread. Retrogradation studies have usually been limited to solutions containing less than 10% of starch which is many times less than that in bread. Schoch (1941) has pointed out that the rate of retrogradation of starch solutions increases with concentration. In spite of the difficulty of highly branched molecules to orient into a crystalline-like structure, aggregation of amylopectin molecules may conceivably occur in bread where the starch is considered to have undergone only a restricted type of gelatinization and where the high gel concentration increases the probability of alignment of the branches.

Summary

Bread was baked from gluten-starch mixtures, the starch component of which consisted of mixtures of wheat starch with various quantities of waxy maize starch. The rate of staling was followed by compression and swelling tests on the crumb.

Flour absorption and dough development time increased, and specific volume and crumb rigidity decreased, with an increase in the percentage of waxy maize starch present. Complete substitution of waxy maize starch for wheat starch gave loaves which were characterized externally by a shiny, sugary crust and internally by a gummy, compressible crumb and excessive shrinkage after baking.

Crumb compressibility and crumb swelling capacity, measured three hours after baking, were increased when the starch of waxy maize was substituted for that of wheat. Upon storage, crumb compressibility of loaves containing waxy maize decreased at about the same rate as that of loaves made from mixtures of gluten and wheat

starch. Swelling capacity of the crumb decreased more rapidly with time when waxy maize starch was present.

These studies suggest that bread staling is associated with changes in the branched chain component (amylopectin) of wheat starch.

Acknowledgment

The authors are indebted to Dr. C. W. Ofelt for preparing the dried gluten and wheat starch used in this study.

Literature Cited

Aitken, T. R., and Anderson, J. A.
1943 Testing the baking strength of gluten concentrates. Cereal Chem. 20: 79–81.
—— and Geddes, W. F.
1938 The effect on flour strength of increasing the protein content by addition of dried gluten. Cereal Chem. 15: 181–196.
1939 The relation between protein content and strength of gluten-enriched flours. Cereal Chem. 16: 223–231.
Alsberg, C. L.
1936 The stale bread problem. Wheat Studies Food Research Inst. 12 (6): 221–247.
Bates, F. L., French, D., and Rundle, R. E.
1943 Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. 65: 142–148.
Brimhall, B., and Hixon, R. M.
1939 The rigidity of starch pastes. Ind. Eng. Chem. (Anal. Ed.) 11: 358–361.
Cathcart, W. H.
1940 Review of progress in research on bread staling. Cereal Chem. 17: 100–121.
—— and Luber, S. V.
1939 Modification of the "swelling power" test for the staling of bread. Cereal Chem. 16: 430–440.
Finney, K. F.
1943 Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20: 381–396.
Foster, J. F., and Hixon, R. M.
1943 Solution viscosities of the amylose components of starch. J. Am. Chem. Soc. 65: 618–622.
Geddes, W. F., and Aitken, T. R.
1935 An experimental milling and baking technique requiring 100 grams wheat. Cereal Chem. 12: 696–707.
Harris, R. H.
1940 A comparative study of some properties of dried glutens prepared from various types of wheat. Cereal Chem. 17: 222–232.
—— and Sibbitt, L. D.
1942 The comparative baking qualities of hard red spring wheat starches and glutens as prepared by the gluten-starch blend baking method. Cereal Chem. 19: 763–772.
Hixon, R. M.
1943 Some recent developments in starch chemistry. Bakers Digest 17 (2): 19–21.
—— and Sprague, G. F.
1942 Waxy starch of maize and other cereals. Ind. Eng. Chem. 34: 959–962.
Katz, J. R.
1928 Gelatinization and retrogradation of starch in relation to the problem of bread staling. In A Comprehensive Survey of Starch Chemistry, edited by R. P. Walton, pp. 100–117. Chemical Catalog Co., New York.
1930 Abhandlungen zur physikalischen Chemie der Stärke und der Brotbereitung. I. Über die Änderungen im Röntgenspektrum der Stärke beim Backen und beim Altbackenwerden des Brotes. Z. physik. Chem. A150: 37–59.

Katz, J. R.
1934 Abhandlungen zur physikalischen Chemie der Stärke und der Brotbereitung. XX. Über den Zusammenhang der Änderung der Stärke beim Altbackenwerden des Brotes und beim Retrogradieren von Stärkekleister. Z. physik. Chem. A169: 321–338.

Meyer, K. H.
1942 Recent developments in starch chemistry. In Advances in Colloid Science, edited by E. O. Kraemer, pp. 143–182. Interscience Publishers, Inc., New York.

Noznick, P. P., and Geddes, W. F.
1943 Application of the Baker compressimeter to cake studies. Cereal Chem. 20: 463–477.

Platt, W., and Powers, R.
1940 Compressibility of bread crumb. Cereal Chem. 17: 601–621.

Radley, J. A.
1943 Starch and Its Derivatives. Chapman and Hall Ltd., London, pp. 103–120.

Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.
1939 Starch in relation to some baking properties of flour. Cereal Chem. 16: 780–792.

Schoch, T. J.
1941 Physical aspects of starch behavior. Cereal Chem. 18: 121–128.
1942 Fractionation of starch by selective precipitation with butanol. J. Am. Chem. Soc. 64: 2957–2961.

—— and Williams, C. B.
1944 Adsorption of fatty acid by the linear component of corn starch. J. Am. Chem. Soc. 66: 1232–1233.

Schopmeyer, H. H., Felton, G. E., and Ford, C. L.
1943 Waxy cornstarch as a replacement for tapioca. Ind. Eng. Chem. 35: 1168–1172.

Whistler, R. L., and Hilbert, G. E.
1945 Separation of amylose and amylopectin by certain nitroparaffins. J. Am. Chem. Soc. 67: 1161–1165.

EFFECT OF SOY FLOUR AND NONFAT DRY MILK SOLIDS IN WHITE BREAD ON THE NUTRITIONAL QUALITY OF THE PROTEIN AS MEASURED BY THREE BIOLOGICAL METHODS

Shirley C. Carlson, F. H. Hafner, and J. W. Hayward

Biological Research and Development, Archer-Daniels-Midland Company,
Minneapolis, Minnesota

(Received for publication January 16, 1946)

The nutritional quality of white bread[1] has received considerable study, especially during the recent war years, and the resulting "enriched bread" program has undoubtedly improved the American diet with respect to the added vitamins and iron. The protein of white bread is another subject receiving considerable attention at present.

---

[1] The terms white bread and whole wheat bread, as used throughout this paper, refer to the products defined in the proposed order of the Federal Security Agency (August 3, 1943). In referring to white bread or whole wheat bread made without milk or other optional proteinaceous materials, the terms white water bread and whole wheat water bread are used.

Numerous experiments, using both human and animal subjects, have demonstrated that the protein of whole wheat flour and bread, in spite of lower digestibility, has superior nutritional quality to that of white flour and white water bread. Publications of the following are cited as examples: Murlin, Marshall, and Kochakian (1941), Rostorfer, Kochakian, and Murlin (1943), Chick (1942), reviews by Boas Fixsen (1935), and Copping (1939). French and Mattill (1935) found no difference in biological value between white and whole wheat breads, but their work is not strictly comparable with the above, since their "whole wheat" bread was 50% white flour and both breads contained an unspecified amount of nonfat dry milk solids.[2]

The chief deficiency of wheat protein is the amino acid, lysine. This was shown as early as 1914 by Osborne and Mendel (1914) and was confirmed by Mitchell and Smuts (1932), using paired feeding. Light and Frey (1943), using ad libitum growth tests with rats, demonstrated that lysine is the primary amino acid deficiency and valine the secondary.

Nonfat milk solids, which supply lysine and valine, have been used rather extensively by the baking industry in white bread in percentages up to 6% of the flour. Fairbanks (1939), using paired feeding, Light and Frey (1943), using ad libitum feeding, and Mitchell, Hamilton, and Shields (1943), using both feeding techniques, have shown that 6% nonfat milk solids effect improvement in the growth-promoting properties of white water bread. This same improvement was effected in white flour in an ad libitum feeding trial by Hove, Carpenter, and Harrel (1945). Mitchell *et al.* (1943) and Light and Frey (1943) found 6% white milk bread [3] equal to whole wheat water bread in their trials.

The protein of soy flour, due to its lysine and valine content, may also be used to increase the nutritional quality of white flour protein. The lysine content of soy protein is 5.4% and that of milk protein is 7.5%; the valine content of both proteins is identical, 4.5%. Defatted soy flour, however, contains about one and one-half times as much protein as nonfat milk solids. Therefore, defatted soy flour actually contributes an equal amount of lysine and more valine than nonfat milk solids when similar percentages of either are incorporated into bread. This is illustrated below:

---

[2] Nonfat dry milk solids will be referred to hereinafter as nonfat milk solids.

[3] The term 6% white milk bread refers to bread made with 100 parts of white flour plus 6 parts of nonfat milk solids. Likewise the term 5% white soy bread means bread made with 100 parts of white flour plus 5 parts of soy flour. Three percent white milk bread and 3% white soy bread are similarly defined.

	Protein %	Lysine %	Valine %
Soy flour, defatted	50.0	2.7	2.3
Nonfat milk solids	35.0	2.6	1.6
White water bread	13.0	0.19	0.33
3% white soy bread	14.5	0.27	0.38
3% white milk bread	13.9	0.27	0.36
5% white soy bread	15.3	0.33	0.41
6% white milk bread	14.8	0.33	0.40

The amino acid figures for the proteins of soy flour, nonfat milk solids, white flour,[4] and bakers' yeast[5] were taken from Block and Bolling (1945) and were used to calculate the amino acid content of the breads.

Earlier nutritional studies on the use of soy flour in bread, reviewed by Horvath (1938), demonstrated the definite supplemental effect of 10% to 30% soy flour. Recently work has been done on the contribution of low percentages of soy flour to the nutritional quality of bread protein.

Jones and Divine (1944), using an ad libitum growth test, compared the supplementary value of several proteins to white flour. They reported their data in the form of both total gain in body weight and protein efficiency.[6] They placed emphasis on the gains in body weight, and so concluded that 5% expeller soy flour was inferior in supplementary value to 5% nonfat milk solids when combined with 95% white flour. However, their results for protein efficiency showed that the 5:95 soy flour and the 5:95 nonfat milk solids mixtures with white flour were equal. It seems more valid to emphasize their protein efficiency data, since the food intake was not equalized. The results of Harris, Clark, and Lockhart (1944), who used an ad libitum growth test, showed that a 3% white soy bread made with full-fat soy flour was equal to a 4% white milk bread in the nutritional quality of protein, both breads being superior to white water bread. Their 5% white soy bread was equal to their 6% white milk bread in the same test. In addition, they found a bread containing both 3% full-fat soy flour and 3% nonfat milk solids superior to either 5% white soy bread or 6% white milk bread. Volz, Forbes, Nelson, and Loosli (1945), using both a paired feeding growth test and nitrogen balance technique, showed that 5% full-fat soy flour improved the protein efficiency and biological value of the protein of 3% white milk bread.

Biological methods for measuring differences in nutritional quality between proteins from various sources are of two types: nitrogen balance studies, in which dietary nitrogen retention is the basis for evaluating proteins, and animal growth studies, in which body protein gain and protein efficiency may be taken as the measure. Two methods of feeding have been used in the latter type of test—ad libitum and equalized. All methods have been subject to criticism and their limitations and advantages have been discussed by Barnes, Maack, Knights, and Burr (1945), and Mitchell (1944), authorities representing different points of view.

---

[4] 1.9% lysine and 3.4% valine in white flour protein.

[5] 6.4% lysine and 4.4% valine in bakers' yeast protein.

[6] The term protein efficiency is used in this paper to designate grams gain in weight per gram of protein eaten, sometimes referred to as nutritive value of protein.

The purpose of the experiments reported herein was to determine by the various biological methods the nutritional quality of the protein in white water bread, white bread containing low levels of soy flour, white bread containing similar levels of nonfat milk solids, and whole wheat water bread. This procedure was adopted in order to establish reliability of the results, in view of the differences of opinion on methods of evaluating the nutritional quality of food proteins.

Materials and Methods

Three experiments were included in this investigation. Experiment I was a growth assay using ad libitum feeding. Experiment II was a growth assay in which food intake was equalized. Body protein storage measurements were included as part of the latter assay. Experiment III was a nitrogen balance study.

Six types of experimental breads were used: white water bread, 3% white soy bread, 5% white soy bread, 3% white milk bread, 6% white milk bread, and whole wheat water bread. All breads except the whole wheat water bread were made from enriched flour.[7] The experimental breads were incorporated into the diets at levels to provide 10% protein (N × 6.25).[8] These diets, formulated for nutritional completeness with respect to all known nonprotein nutrients, derived their protein solely from the breads.

Preparation of the Breads. The breads used in these studies were prepared in laboratory equipment using a straight-dough mix. The formula consisted of enriched white flour (80% patent) 100 parts, compressed yeast 2 parts, sugar 3 parts, salt 2 parts, shortening 2 parts, Arkady [9] 0.45 part, and water as determined by absorption.[10] The stipulated number of parts of soy flour and nonfat milk solids was added to 100 parts of enriched white flour.

The enriched white flour and the whole wheat flour were made from the same blend of hard spring wheat. The enriched white flour was bleached with Novadel (active principle, benzoyl peroxide) and Agene (nitrogen trichloride) according to commercial practice. The soy flour was of the defatted[1] type and the nonfat milk solids were a spray-dried product; both were made expressly for bakery use. The analysis of these bread ingredients is given in Table I.

---

[7] The enrichment complied with minimum requirements as published by the Federal Security Agency (July 3, 1943), which are. thiamine 2.0 mg, riboflavin 1.2 mg, niacin 16 mg, iron 13 mg per pound of flour.

[8] Since there was no one factor appropriate for all the breads tested, the 6.25 factor was used in all calculations involving conversion of Kjeldahl N to protein in this paper, as in the publication of Hove *et al.* (1945).

[9] A mixture of NH_4Cl, $CaSO_4$, $KBrO_3$, and a starch filler used as a dough improver.

[10] Enriched white flour had an absorption of 66%. The other flour mixes had higher absorptions

TABLE I

ANALYSIS OF BREAD INGREDIENTS

Ingredient	Type	Moisture	Protein	Fat[1]	Ash
		%	%	%	%
Enriched white flour	Hard spring	13.1	12.8	0.9	0.4
Nonfat milk solids	Spray dried	5.3	37.1	0.7[2]	—
Soy flour	Defatted	7.3	50.6	1.2	5.8
Whole wheat flour	Hard spring	12.1	15.3	2.1	1.8

[1] Ether extract following acid hydrolysis. [2] Rohrig tube method of Roese-Gottlieb.

The doughs were mixed in a Hobart Mixer for 1 minute at low speed and for 4 minutes at medium speed. When delivered from the mixer, they had an average temperature of 80°–82°F. The fermentation schedule was first punch 1½ hours, to bench ¾ hour, to molder ¼ hour; total fermentation time 2½ hours at 80°F. The fermented doughs were panned, proofed 60 minutes at 90°F, and baked 30 minutes at 425°F in a rotary-hearth type oven. The freshly baked breads were sliced, air-dried, and ground to a powder of approximately 80 mesh. Table II contains the analysis of the dried breads as they were used in the diets.

TABLE II

ANALYSIS OF DRIED BREADS

Description of breads			Moisture	Protein	Fat[2]	Ash
Bread flour	Protein supplement					
		Parts[1]	%	%	%	%
Enriched white	None		9.6	13.0	3.0	2.5
Enriched white	Nonfat milk solids	3	8.4	13.9	3.3	2.6
Enriched white	Nonfat milk solids	6	7.5	14.8	3.4	2.6
Enriched white	Soy flour	3	7.0	14.5	3.5	2.6
Enriched white	Soy flour	5	6.9	15.3	3.0	2.6
Whole wheat	None		7.2	14.2	3.6	2.9

[1] Parts added to 100 parts of white flour [2] Ether extract following acid hydrolysis.

Preparation of the Diets. The diets were formulated according to the following basic pattern: dried bread to supply 10% protein, minerals[12] 4.0%, cod liver oil 2.0%, celluration[13] to equalize crude fiber at approximately 2%, soybean oil to equalize fat at 6%, and corn starch to make up the balance of the diets. In Experiment I, 4.0% of B-complex vitamin solution[14] was incorporated into the diets. In Experiments II and III each rat received orally 0.4 ml of the B-complex vitamin solution every day. Mixtures of dry ingredients, equivalent to 6 kilograms of complete diet, were prepared for each lot. Kilogram quantities were mixed with the liquid ingredients as needed. Protein analyses of the complete diets are given in column 2 of Table III. The diets were compounded according to the same formula throughout the investigation.

---

[1] Hexane extracted.

Feeding and Experimental Procedures. The rats used for all tests were weanling male albinos of the Sprague-Dawley strain, 24 to 25 days old and weighing 40 to 55 g. Within any one of the three assays to be described the animals did not vary more than 10 g in weight. They were individually caged and given access to water. Cages were located in a well-lighted, air-conditioned animal room. Temperature was maintained at 75° ± 2°F at a relative humidity of 50% to 70%. The procedure for each of the experiments is described below:

I. *Growth Assay—Ad Libitum Feeding.* This experiment was a 56-day growth assay using a modification of the method of Osborne, Mendel, and Ferry (1919). Thirty-six rats were distributed at random into six lots of six animals each. Each animal was weighed during the same hour on three consecutive days at the start to obtain average initial weight, and at 7-day intervals for 8 weeks thereafter. Final weights were determined in the same manner as were the initial weights.

The diets were offered ad libitum in cups designed to minimize spillage. The food was weighed fresh each day, and the rejected food from the previous feeding was discarded. Individual food consumption records were averaged at 7-day intervals for each lot. The total weight of protein consumed per rat during the 56-day assay period was determined by multiplying the total feed consumed by the actual percentage of protein in the diet.

II. *Growth Assay—Equalized Intake.* Technique for this assay was the same as described above, except that the total food intake of all animals in this assay was equalized with that of the rat which consumed the least, provided that this rat was not obviously abnormal. This assay was conducted for 63 days. Protein efficiency data were obtained at the end of 56 days; body protein storage was determined at the end of 63 days.

Body Protein Storage. To obtain analytical data on the body protein gains of the rats, the carcass of each animal was opened, the digestive tract removed, and washed free of its contents. The unground carcasses and digestive tracts of the six animals in each lot were then digested in about 3,000 ml of 20% H_2SO_4 until a uniform solution resulted. Digests were diluted to volume and aliquots analyzed for total nitrogen content as described in A.O.A.C. *Official Methods of Analysis* (1940). Eleven weanling males were analyzed by the above procedure to obtain the initial average protein content of the rats; body protein gains were calculated on the basis of these determinations.

---

[12] Osborne-Mendel-Wesson Salt Mixture (Wesson, 1932) modified to contain 0.13 g of $ZnSO_4 \cdot 7\ H_2O$ per kilogram.

[13] Processed rice hulls, analyzing approximately 65–70% crude fiber and 0.0% protein and purchased from Fisher Scientific Company, Chicago, Illinois.

[14] Thiamine, riboflavin, and pyridoxine 300 µg each, niacin 2.5 mg, calcium pantothenate 1.1 mg, para-aminobenzoic acid 7.5 mg, and choline hydrochloride 16.6 mg dissolved in 4 ml of water and used on the basis that 4 ml is equal to 4 g.

TABLE III

SUMMARY OF EXPERIMENTAL DATA AND RESULTS

Diet No.	Protein (N×6.25) in diet	Source of dietary protein			Growth studies[5]							Nitrogen balance study			
					Ad libitum intake			Equalized intake				True digestibility		Biological value	
		Bread flour	Protein supplement		Weight gain	Protein eaten	Protein[6] efficiency	Weight gain	Protein eaten	Protein[6] efficiency	Body protein gain[7]	Part A	Part B	Part A	Part B
	%			*Parts*[2]	g	g		g	g		g	%	%	%	%
392	10.8	White[1]	None		36.0	41.6	0.87	19.1	29.0	0.66	4.2	93.8	92.9	39.2	38.9
393	10.4	White[1]	N.M.S.[3]	3	42.6	42.3	1.01	23.7	28.6	0.83	5.0		94.6		45.6
394	10.4	White[1]	N.M.S.[3]	6	47.7	44.7	1.07	25.9	28.6	0.91	6.0		94.2		49.0
401	10.4	White[1]	Soy flour[4]	3	44.6	44.5	1.00	24.5	28.6	0.85	5.2		93.6		46.5
402	10.2	White[1]	Soy flour[4]	5	58.3	47.1	1.24	26.3	28.6	0.93	5.9	91.7		50.6	
411	10.4	Whole wheat	None		55.5	47.2	1.18	21.5	28.5	0.75	4.2		92.9		45.9
Pooled standard error of means							0.032 N=6			0.042 N=6	0.352 N=6			1.25 N=8	1.43 N=10
Least significant difference between any two means:			Odds 19 : 1				0.12			0.15	1.28			4.19	4.57
			Odds 99 : 1				0.19			0.23	2.01			6.19	6.57

---

[1] Enriched.
[2] Parts added to 100 parts of white flour.
[3] Nonfat milk solids.
[4] Defatted type.
[5] Basis 56 days.
[6] Grams gain in weight per gram of protein eaten, sometimes referred to as nutritive value of protein.
[7] Basis 63 days.

III. Nitrogen Balance Test. The biological value and digestibility for each protein were determined using the technique of Mitchell and Carman (1926), with modifications described by Mitchell (1943). The nitrogen balance experiment was carried out in two parts. Part A, using eight rats, was a comparison between 5% white soy bread and white water bread. This test involved an initial standardization period during which a nitrogen-free ration (0.062% N) was fed to all rats for a 4-day preliminary period, and for a 3-day test period in which urine and feces were collected. The animals were then divided into two groups of four rats each, and each group was placed on one of the test rations for a 4-day preliminary and a 7-day collection period. Following this, the diets were reversed for a similar period. Food intake was equalized throughout the test in the same manner as for the growth test with equalized feeding.

Part B was a comparison of white water bread, 3% white soy bread, 3% white milk bread, 6% white milk bread, and whole wheat water bread. Biological values for the protein in these breads were determined using 10 rats for each bread. This test was conducted in the same manner as Part A, except that a final nitrogen-free period, similar to the initial one, was run after the two periods in which the test diets were fed. An average of initial and final endogenous nitrogen was used in calculating biological values. This variation was incorporated to reduce the relatively high endogenous nitrogen, and subsequently higher biological values obtained when using only an initial nitrogen-free period, as reported by Olson and Palmer (1940). White water bread was included in both Parts A and B so that a comparison of values by both procedures could be made.

Results and Discussion

The results of all the experiments are shown in Table III.[15] Statistical analyses of the data were made by the method of Snedecor (1940), using covariance analysis for the ad libitum growth study and analysis of variance for both evaluations of the equalized feeding assay. Brandt's method of testing significance in reversal experiments, described by Snedecor, was used for the nitrogen balance test.

All the methods used showed that the proteins of the two white soy breads and the two white milk breads were superior in nutritional quality to the protein of white water bread. As measured by the biological values and the protein efficiencies from both growth assays, the differences between white soy or white milk breads and white water bread were in all cases statistically significant. The body protein gains, food intake being equalized, were significantly higher for 5% white soy and 6% white milk breads than for white water bread; the body protein gains for 3% white soy and 3% white milk breads were also higher than for white water bread, although these latter dife ferences were not statistically significant.

The 3% white soy bread was equal in the nutritional quality of the protein to 3% white milk bread, both breads being superior to whit- water bread, as determined by all three experimental methods. Our results for these breads are given below with the results of other investigators:

---

[15] Complete data for the nitrogen balance test may be obtained from the authors on request.

	Biological value			Protein efficiency				Body protein gains
				Ad libitum intake		Equalized intake		
	This paper	Henry *et al.* (1941)	Volz *et al.* (1945)	This paper	Harris *et al.*[1] (1944)	This paper	Volz *et al.* (1945)	This paper
	%	%	%					g
Bread								
White water bread	39.2 (A) 38.9 (B)	44.7		0.87	0.51	0.66		4.2
2% White milk bread	—	47.6		—	—	—		—
3% White milk bread	45.6		43.3	1.01	—	0.83	0.97	5.0
3% White soy bread	46.5			1.00	0.69	0.85		5.2
4% White milk bread	—			—	0.67	—		—
				Jones & Divine (1944)	Hove *et al.* (1945)			
Flour								
White flour				0.75	0.84		0.80	
White flour, 97 parts +N.M.S.,[2] 3 parts					1.00			

[1] The values reported by Harris *et al.* (1944) were converted to protein efficiency values by dividing by the factor 6.25 for convenience in comparing data.

[2] Nonfat milk solids.

The protein of 5% white soy bread was found equal to the protein of 6% white milk bread in nutritional quality when comparing biological values, protein efficiencies, and body protein gains, using equalized feeding. The protein efficiencies for these same breads in the ad libitum assay showed 5% white soy bread to be significantly better than 6% white milk bread, although neither bread differed significantly from whole wheat water bread. A comparison of our results with those reported in the literature is tabulated on page 314.

Since our protein efficiency results for 5% white soy bread and 6% white milk bread in the ad libitum feeding test do not agree with those reported by Jones and Divine (1944) with flours or Harris *et al.* (1944) with breads, the possibility that heat of baking may have had some effect on the performance of soy and milk proteins is receiving consideration.

In any of the experiments included in our study, the nutritional quality of the protein in either 5% white soy or 6% white milk bread did not differ statistically from that in either 3% white soy or 3% white milk bread, with one exception. This was in the ad libitum growth assay in which the protein efficiency of 5% white soy bread was significantly higher than that of the other white breads. However, biological values and protein efficiencies from both growth assays showed that there was a significant (odds 19:1) difference between 3%

	Biological value			Protein efficiency				Body protein gains
				Ad libitum intake		Equalized intake		
	This paper	Henry *et al* (1941)	Volz *et al* (1945)	This paper	Harris *et al* (1944)	This paper	Volz *et al.* (1945)	This paper
	%	%	%					g
Bread								
5% White soy bread	50.6			1.25	0.75	0.93		5.9
6% White milk bread	49.0	49.7		1.07	0.76	0.91	—	6.0
White (3%) milk (5%) soy bread	—		47.7	—		—	1.17	—
Whole wheat water bread	45.9			1.18		0.75	-	4.2
				Jones & Divine (1944)	Hove *et al.* (1945)	Barnes *et al* (1945)		
Flour								
White flour, 95 parts +Soy flour, 5 parts				1.38				
White flour, 95 parts +N.M.S.,[1] 5 parts				1.44			1.13	
White flour, 94 parts +N.M.S.,[1] 6 parts					1.19			
Whole wheat flour				1.15	1.40	0.42		

[1] Nonfat milk solids.

white soy or 3% white milk bread and white water bread proteins, while there was a highly significant (odds 99:1) difference between 5% white soy or 6% white milk bread and white water bread proteins. The body protein storage data showed this same trend. Therefore, our results by all biological methods showed that the nutritional quality of the protein in white bread increased as the percentage of soy flour or nonfat milk solids added to the bread was increased from 3% to 5% or 6%.

The biological values of the proteins showed whole wheat water bread to be superior to white water bread, equal to 3% white soy or 3% white milk bread, but slightly inferior to 5% white soy or 6% white milk bread. In our ad libitum growth assay, the protein efficiency of whole wheat water bread was significantly higher than the protein efficiencies of white water bread, 3% white soy, or 3% white milk bread, but did not differ significantly from those of 5% white soy or 6% white milk bread. However, in the growth assay employing equalized food intake, the protein efficiencies and body protein gains for whole wheat water bread did not differ significantly from those for white water bread. Under the same conditions, the protein efficiencies and body protein gains of whole wheat water bread were slightly lower than those of 3% white milk or 3% white soy bread, and significantly lower than those of 6% white milk or 5% white soy bread.

These results are in contrast to those of Mitchell *et al.* (1943) who, in their paired feeding assay as well as in their ad libitum assay, found equal growth-promoting value for whole wheat water bread and 6% white milk bread, white water bread being inferior. They were, however, feeding the entire breads plus cod liver oil and hence a higher, as well as a more variable, protein level than was used in our study. The protein analyses for their white water bread, 6% white milk bread, and whole wheat water bread were 14.06%, 14.75%, and 15.75%, respectively.

Our results are in agreement with those of Barnes *et al.* (1945) who reported a lower protein efficiency for whole wheat flour when food intake was restricted than when their animals were fed ad libitum. In their trial, the food intake of each rat was restricted by paired feeding for 54 days and then a period of ad libitum feeding was begun. The results we obtained for whole wheat water bread in our ad libitum and equalized feeding trials confirm their results as shown below:

	Protein in diet	Type of feeding	Daily food intake	Protein efficiency
	%		g	
This paper	10.4	Restricted	5.1	0.75
		Ad libitum	7.4	1.18
Barnes *et al.* (1945)	11.2	Restricted	3.44	0.42
		Ad libitum	7.46	1.03

The nutritional quality of the protein of whole wheat water bread apparently varies with the type of feeding and the level of protein in the diet. In our assay the use of different biological methods resulted in a change in relative rank of the protein in whole wheat water bread when compared with the proteins in the other breads as to nutritional quality. Whole wheat protein probably does not furnish sufficient amino acids for growth when the food intake is considerably restricted on a low (10%) protein diet.

Summary

Three biological methods were used to evaluate the nutritional quality of the protein present in white water bread, 3% and 5% white soy breads, 3% and 6% white milk breads, and whole wheat water bread. The three biological methods used were as follows: nitrogen balance; two growth assays, one using ad libitum and the other equalized food intake; and body protein storage.

The results for the nutritional quality of the bread protein obtained by the three methods showed that 3% white soy bread and 3% white milk bread were equal, and that both of these breads were significantly better than white water bread. The 5% white soy bread was at least equal to 6% white milk bread, and both of these were slightly superior to the 3% white soy or 3% white milk breads, and significantly superior to white water bread. The 5% white soy bread was equal to or better than whole wheat water bread, as was 6% white milk bread, except in the ad libitum growth assay where the difference was not significant.

The three methods showed good agreement with respect to the relative rank of the five white bread proteins, while the relative position of whole wheat water bread protein was apparently related to the level of food intake.

Acknowledgments

The authors are indebted to the following people of our company: W. L. Ramey and R. M. Bohn, for the preparation of the experimental breads used in these studies, Elizabeth C. Herrmann, for help in the preparation of the manuscript; and Royce E. Anderson, for analyses of the materials. We also wish to thank Raymond E. Carlson, formerly of our company, for part of the experimental work.

Literature Cited

Association of Official Agricultural Chemists
1940 Official Methods of Analysis II, 24: 26-27.
Barnes, R. H., Maack, J. E., Knights, M. J., and Burr, G. O.
1945 Measurement of the growth-promoting quality of dietary protein. Cereal Chem. 22: 273-286.
Block, R. J., and Bolling, D.
1945 The Amino Acid Composition of Proteins and Foods. Charles C. Thomas, Springfield, Illinois.
Boas Fixsen, M. A.
1935 The biological value of protein in nutrition. Nutrition Abstracts & Revs. 4: 447-459.
Chick, H.
1942 Biological value of the proteins contained in wheat flour. Lancet 242: 405-407.
Copping, A. M.
1939 The nutritive value of wheaten flour and bread. Nutrition Abstracts & Revs. 8: 555-566.
Fairbanks, B. W.
1939 A study by the paired feeding method of the nutritive value of bread made with milk solids. Cereal Chem. 16: 404-414.
Federal Security Agency
1943 July 3. Wheat flour and related products. Amendments to definitions and standards of identity. Federal Register 8: 9115.
1943 August 3. Various kinds of bread. Definitions and standards of identity. Federal Register 8: 10780-10788.
French, R. B., and Mattill, H. A.
1935 The biological value of the proteins of white, wheat, and rye breads. Cereal Chem. 12: 365-371.
Harris, R. S., Clark, M., and Lockhart, E. E.
1944 Nutritional value of bread containing soya flour and milk solids. Arch. Biochem. 4: 243-247.

Henry, K. M., Houston, J., Kon, S. K., Powell, J., Carter, R. H., and Halton, P.
1941 The effects of additions of dried skim milk and dried whey on the baking quality and nutritive properties of white bread. J. Dairy Research **12:** 181–212.

Horvath, A. A.
1938 The nutritional value of soybeans. Am. J. Digestive Diseases Nutrition **5:** 177–183.

Hove, E. L., Carpenter, L. E., and Harrel, C. G.
1945 The nutritive quality of some plant proteins and the supplemental effect of some protein concentrates on patent flour and whole wheat. Cereal Chem. **22:** 287–295.

Jones, D. B., and Divine, J. P.
1944 The protein nutritional value of soybean, peanut, and cottonseed flours and their value as supplements to wheat flour. J. Nutrition **28:** 41–49.

Light, R. F., and Frey, C. N.
1943 The nutritive value of white and whole wheat breads. Cereal Chem. **20:** 645–660.

Mitchell, H. H.
1943 Biological methods of measuring the protein values of feeds. J. Animal Sci. **2:** 263–277.
1944 Determination of the nutritive value of the proteins of food products. Ind. Eng. Chem. (Anal. Ed.) **16:** 696–700.

———, and Carman, G. G.
1926 The biological value of the nitrogen of mixtures of patent white flour and animal foods. J. Biol. Chem. **68:** 183–215.

———, Hamilton, T. S., and Shields, J. B.
1943 The contribution of non-fat milk solids to the nutritive value of wheat breads. J. Nutrition **25:** 585–603.

———, and Smuts, D. B.
1932 The amino acid deficiencies of beef, wheat, corn, oats, and soy beans for growth in the white rat. J. Biol. Chem. **95:** 263–281.

Murlin, J. R., Marshall, M. E., and Kochakian, C. D.
1941 Digestibility and biological value of whole wheat breads as compared with white bread. J. Nutrition **22:** 573–588.

Olson, F. C., and Palmer, L. S.
1940 Comparison of a chemical and a biochemical method for determining the biological value of proteins and an evaluation of the endogenous nitrogen. J. Agr. Research **60:** 331–342.

Osborne, T. B., and Mendel, L. B.
1914 Amino-acids in nutrition and growth. J. Biol. Chem. **17:** 325–349.

———, ———, and Ferry, E. L.
1919 A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem. **37:** 223–229.

Rostorfer, H. H., Kochakian, C. D., and Murlin, J. R.
1943 Digestion of whole wheat and white breads in the human stomach. J. Nutrition **26:** 123–138.

Snedecor, G. W.
1940 Statistical Methods. Iowa State College Press, Ames, Iowa.

Volz, F. E., Forbes, R. M., Nelson, W. L., and Loosli, J. K.
1945 The effect of soy flour on the nutritive value of the protein of white bread. J. Nutrition **29:** 269–275.

Wesson, L. G.
1932 A modification of the Osborne-Mendel salt mixture containing only inorganic constituents. Science **75:** 339–340.

THE VITAMIN B-COMPLEX CONTENT OF THE COMMERCIAL MILLING PRODUCTS OF EGYPTIAN RICE

STANLEY A. PRICE

Research Laboratories, Vitamins Ltd., London, England

(Received for publication October 29, 1945)

Kik and Van Landingham (1943, 1944), Williams, Knox, and Fieger (1943), and Kik and Williams (1945) have published extensive figures for the vitamin B-complex content of many varieties of rice grown and milled in the principal rice-producing localities of the United States. No similar data appear to be available for Egyptian rice, and the present investigation was undertaken at the suggestion of the Ministry of Food in September, 1944. Interest lay primarily in the distribution of the vitamins of the B_2 complex, but a few figures for thiamine (Vitamin B_1) were also obtained.

Materials and Methods

The paddy used was Egyptian-grown from a hybridized Japanese-type seed. With the exception of the tail-ends, all the samples were drawn from a single bulk mill lot of 12.5 tons of paddy.

Thiamine was determined by the method of Harris and Wang (1941).

Pyridoxine was determined by the method of Stokes *et al.* (1943), using the "pyridoxinless" mutant (No. 299) of *Neurospora sitophila* as test organism.

Riboflavin was determined by the method of Snell and Strong (1939), using *Lactobacillus helveticus*, as modified by Barton-Wright and Booth (1943). Extracts were prepared by autoclaving 1 to 5 g of the finely ground material with 50 ml 0.1 *N* hydrochloric acid for 15 minutes at 15 pounds per square inch, followed by precipitation and filtration at pH 4.5 as recommended by Strong and Carpenter (1942). This latter modification is essential for the assay of cereals with the media at present in use, as it minimizes the "drift" so frequently observed in the assay of substances of this type. Even so, some non-specific growth-stimulation is frequently obtained at the lower riboflavin levels, and it is considered that the technique is still capable of improvement. Precautions were taken to ensure uniformity of incubation temperature, as stressed by Price and Graves (1944), and the tests were incubated for 72 hours in a mechanically stirred water bath at 40 ± 0.05°C.

Nicotinic Acid was determined by a microbiological method using *Lactobacillus arabinosus*. The medium was that described by Snell and Wright (1941), although in subsequent work the modifications proposed by Krehl, Strong, and Elvehjem (1943) have been adopted. Extracts of samples for assay were prepared by autoclaving 1 to 5 g of the finely ground material with 50 ml *N* hydrochloric acid for 15 minutes at 15 pounds per square inch. After cooling, the pH was adjusted to 6.8, the extracts suitably diluted for assay and filtered. In our experience this has proved one of the most satisfactory of the microbiological methods. Agreement between the titrations on duplicate tubes is consistently good, and excellent reproducibility is obtained when the sample is assayed on different occasions.

Results and Discussion

The values obtained are shown in Table I. Since the proportions by weight of the by-products and of the finished rice are known, it is of interest to investigate the distribution, during the milling process, of the vitamins originally present in the paddy. The distribution of nicotinic acid, pyridoxine, and riboflavin is shown in Table II.

TABLE I

VITAMIN B-COMPLEX CONTENT OF RICE MILLING FRACTIONS

Sample	Vitamin content			
	Nicotinic acid	Pyridoxine	Riboflavin	Thiamine
	µg/g	µg/g	µg/g	µg/g
Paddy	50.4	8.1	0.66	—
Loonzain (brown rice)	50.6	—	—	—
Hulls (husks)	9.2	3.6	0.49	—
1st Coning Rice	33.4		—	—
2nd Coning Rice	20.9		—	—
Meal from 1st Coning	506.0	63.0	2.2	34.8
Meal from 2nd Coning	365.0	52.0	2.6	36.9
Fannings[1]	98.8	14.6	1.2	6.9
Dusty chaff[1]	128.0	14.5	1.2	4.2
Brokens[1]	42.6	5.7	0.76	-
Tail ends	57.5	5.5	0.55	—
Finished rice	17.6	3.4	0.35	—

[1] The terms "fannings" and "dusty chaff" are colloquialisms used by the miller for by-products composed mainly of meal and fine husks. "Brokens" includes both the "screenings" and "brewer's rice" of American milling practice

The results are in general agreement with those for American-grown rice published by Kik and Van Landingham (1943 and 1944) and Williams, Knox, and Fieger (1943). Our value for the nicotinic acid content of hulls (9.2 µg/g), however, is lower than that found by

TABLE II

DISTRIBUTION OF B-COMPLEX VITAMINS IN THE RICE MILLING PROCESS

Sample	% by weight of paddy	Contribution made by each fraction per 100 g of paddy			% of original vitamins retained in each fraction		
		Nicotinic acid	Pyridoxine	Riboflavin	Nicotinic acid	Pyridoxine	Riboflavin
	%	µg	µg	µg	%	%	%
Paddy	100	5040	810	66	100	100	100
Hulls (husks)	16.01	148	58	7.8	2.9	7.2	11.8
Meal from 1st Coning	3.3	1670	208	7.3	33.1	25.7	11.1
Meal from 2nd Coning	3.12	1139	162	8.1	22.6	20.0	12.3
Fannings	1.36	134	20	1.6	2.7	2.5	2.4
Dusty chaff	1.36	174	20	1.6	3.5	2.5	2.4
Brokens	3.32	142	19	2.5	2.8	2.3	3.8
Tail ends [1]	7.72	443	42	4.2	8.6	5.2	6.4
Finished rice	60.88	1072	207	21.3	21.5	25.6	32.3
Weight loss on milling	2.93						
Totals	100	4922 (97.7%)	736 (91.0%)	54.4 (82.5%)	97.7	91.0	82.5

[1] This sample derived from a different mill lot.

Kik and Van Landingham (1944), who reported 14.0–25.1 µg/g. Our nicotinic acid figures for the first and second coning meals (506 and 365 µg/g) are higher than these authors' findings (303.2–358.2 and 259.5–316.0 µg/g); they approximate more closely those of Williams, Knox, and Fieger (1943) for rice bran (334.8–487.4). We find higher thiamine and pyridoxine values (34.8 and 36.9 µg thiamine per gram and 63 and 52 µg pyridoxine per gram) than were reported by Williams *et al.* (1943) for rice bran (26.6–30.1 µg thiamine per gram and 23.8–38.6 µg pyridoxine per gram). Kik and Van Landingham (1943) report only 0.25–0.41 and 0.25–0.49 µg riboflavin per gram in screenings and brewer's rice respectively, while 0.76 µg/g was found for broken rice in the present work.

The riboflavin is more evenly distributed throughout the grain than is the nicotinic acid; pyridoxine is intermediate in this respect. A similar observation regarding the distribution of nicotinic acid and riboflavin in wheat has been made by Jackson, Doherty, and Malone (1943).

The apparent difference between the total riboflavin content of the various fractions in Table II and the riboflavin content of the whole paddy is within the limits of experimental error for samples of such relatively low potency. The "weight loss" (2.93%) is abnormally high, owing to the fact that the samples were drawn on the first day of milling.

Summary

The nicotinic acid, pyridoxine, and riboflavin contents of Egyptian paddy and milling fractions are in substantial agreement with figures published for rice grown and milled in the United States.

Of the nicotinic acid, pyridoxine, and riboflavin present in the original paddy, 21.5, 25.6, and 32.3% of these factors respectively were retained in the finished rice, and 55.7, 45.7, and 23.4% were removed as rice meal.

Acknowledgments

I am indebted to the Rice Division, Ministry of Food, which, through Messrs. Carbutt and Company (1928) Ltd., kindly provided the samples for assay. In particular my thanks are due to Mr. J. McCraken for his expert advice, and to my colleagues, Mr. J. V. Croucher and Dr. R. Poplett, who carried out the thiamine and pyridoxine assays.

Literature Cited

Barton-Wright, E. C., and Booth, R. G.
1943 The assay of riboflavin in cereals and other products. Biochem. J. 37: 25–30.
Harris, L. J., and Wang, Y. L.
1941 An improved procedure for estimating vitamin B_1 in foodstuffs and biological materials by the thiochrome test including comparisons with biological assays. Biochem. J. 35: 1050–1067.
Jackson, S. H., Doherty, A., and Malone, V.
1943 The recovery of the B vitamins in the milling of wheat. Cereal Chem. 20: 551–559.
Kik, M. C., and Van Landingham, F. B.
1943 Riboflavin in products of commercial rice milling and thiamin and riboflavin in rice varieties. Cereal Chem. 20: 563–569.
1944 Nicotinic acid in products of commercial rice milling and in rice varieties. Cereal Chem. 21: 154–158.
—— and Williams, R. R.
1945 The nutritional improvement of white rice. Bull. Natl. Research Council No. 112.
Krehl, W. A., Strong, F. M., and Elvehjem, C. A.
1943 Determination of nicotinic acid. Ind. Eng. Chem. (Anal. Ed.) 15: 471–475.
Price, S. A., and Graves, H. C. H.
1944 Microbiological assay of riboflavin. Nature 153: 461.
Snell, E. E., and Strong, F. M
1939 A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.) 11: 346-350.
—— and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. 139: 675–686.
Stokes, J. L., Larsen, A., Woodward, C. R., and Foster, J. W.
1943. A Neurospora assay for pyridoxine. J. Biol. Chem. 150: 17–24.
Strong, F. M., and Carpenter, L. E.
1942 Preparation of samples for microbiological determination of riboflavin. Ind. Eng. Chem. (Anal. Ed.) 14: 909–913.
Williams, V. R., Knox, W. C., and Fieger, E. A.
1943 A study of some of the vitamin B-complex factors in rice and its milled products. Cereal Chem. 20: 560–563.

THE QUANTITATIVE DETERMINATION OF MOLDS IN FLOUR [1, 2]

CLYDE M. CHRISTENSEN

Division of Plant Pathology and Botany,
University Farm, St. Paul, Minnesota

(Received for publication January 21, 1946)

Methods to determine the number of microorganisms in flour have been described by Kent-Jones and Amos (1940), and by Barton-Wright (1938), and have been included in *Cereal Laboratory Methods* (4th ed., 1941). These methods were designed principally to assay the number of bacteria, and while they presumably would apply equally well to the molds, there has been almost no attempt to determine whether they actually would permit an accurate mold census to be made of a given flour sample.

The present study was undertaken to determine some of the chief factors that influence the number of molds cultured from a given flour sample. Preliminary work with samples taken from different streams in different mills, and with sterile flour to which known molds were added, indicated that the most important variables involved were: the composition of the medium on or in which the flour was cultured; the technic of making the dilutions; and the method of counting the number of colonies in the culture dishes. Therefore most of our efforts were concentrated on these three variables.

Experimental

Culture Media. About 10 culture media were tested, the following ones fairly extensively: Smith-Humfeld agar, designed by Smith and Humfeld (1930) to facilitate the isolation of a large variety of organisms from soil; a penicillin assay medium essentially the same as the Medium No. 1 described by Schmidt and Moyer (1944) but containing also 1.5 g of beef extract per liter; potato dextrose agar; potato dextrose agar acidified by the addition of 0.5 ml of 50% lactic acid per 100 ml of agar just before pouring into plates; malt agar containing 2% malt extract and 2% agar per liter; and a malt-salt agar made as follows:

Malt extract (any of the several commercial brands tested were suitable)	20.0 g
Sodium chloride	75.0 g
Agar, crude shredded	20.0 g
Distilled water to make	1.0 liter

---

[1] Paper No. 2279, Scientific Journal Series, Minnesota Agricultural Experiment Station. Cooperative investigation between the Division of Biochemistry and the Division of Plant Pathology and Botany.

[2] This investigation was aided by a grant from Wallace and Tiernan Company, Inc., Newark, New Jersey.

The details of preparation of this medium are given later in the paper. It is somewhat acid (about pH 5), and the salt concentration is high enough to inhibit completely the growth of any bacteria so far encountered in flour. Common species of *Rhizopus*, *Mucor*, *Alternaria*, *Penicillium*, and *Aspergillus* grow relatively slowly upon it. Such common and abundant inhabitants of flour as *Aspergillus candidus* and *A. glaucus* grow more rapidly and appear in greater numbers on the malt-salt medium than upon any of the other media tested.

Two flour samples, one containing a relatively small number of a few species of molds, the other containing a relatively large number of a greater variety of molds, were assayed on these five media, using the method described in detail later in the paper. The results are given in Table I.

TABLE I

INFLUENCE OF THE CULTURE MEDIUM ON THE NUMBER OF MOLDS CULTURED FROM TWO SAMPLES OF FLOUR [1]

Medium	Mold colonies per gram of flour Sample 1	Sample 2
Penicillin assay medium	160	— [2]
Potato-dextrose	320	960
Smith-Humfeld	550	3800
Acid potato-dextrose	1180	3800
Malt-salt	1800	5900

[1] Each figure is an average of five plates.
[2] Almost no molds appeared on this agar; all five plates were overgrown with bacteria.

Similar results were obtained in other tests. In most cases a given sample of flour yielded 50% more mold colonies on malt-salt agar than on Smith-Humfeld or acid potato-dextrose agar, and often yielded 1000% more than upon the penicillin assay medium or ordinary potato-dextrose agar. These results suggest that to assay the molds in flour it is necessary to use a medium on which the majority of the molds present will grow better than the other organisms present in the flour. The malt-salt agar seems to fill this requirement for the more than 60 flour streams we have sampled. This does not necessarily mean that it will be the best medium for assaying molds in all flour streams in all mills at all times. A slightly larger number of mold species have been cultured from certain flour streams on Smith-Humfeld agar than on malt-salt agar, but the malt-salt medium has consistently yielded a greater total number of mold colonies.

Consistency of mold count among replicate plates, or between assays of the same flour sample on successive days is not necessarily indicative of the suitability of the medium. The same sample of first break flour was assayed each day for 10 successive days on Smith-Humfeld agar. Each assay consisted of four replicate plates. In most of these assays the flour was diluted 1:100, but some dilutions of 1:25 and 1:50 were also used, to permit a comparison of dilutions. An average of 550 molds per gram was obtained from the total of 40 plates. Five of the four-plate tests averaged between 550 and 575 molds per gram of flour. The number of molds on any one plate fell mostly within ±10% of the general average, and usually the variation was less than ±5%. This was considered very satisfactory replicability. However, the same flour sample cultured by the same method on malt-salt agar yielded an average of approximately 1800 molds per gram.

Comparison of Different Methods of Sampling. Three methods of sampling were tested:

1. That described in detail later in the paper.
2. The flour sample to be assayed was diluted as in Method 1, and 1-ml aliquots of the suspended flour were flooded on the surface of a hardened agar medium. Best results with this method were obtained when the medium was prepared and poured into plates at least a week in advance of use. In that time the medium lost enough water to take up the 1 ml of liquid flooded over it within a few minutes.
3. A dry sampling method. For this, a small metal cup was welded to a wire inoculating needle. The cup was sterilized by flaming, cooled, and filled by pushing it through the flour. Tapping the wire lightly, with the cup inclined at a slight angle, served to remove excess flour from the surface of the cup and insure that it was level full. In numerous tests, the amount of flour delivered by this cup varied from 9.5 to 10.5 mg.

Enough sterile, dry quartz sand was poured into a sterile Petri dish to cover the bottom sparsely. A 10-mg cup of flour was added, and the dish shaken until the sand had distributed the flour particles evenly over the bottom of the dish. The cover was then removed from this dish, and from a Petri dish containing the agar medium. The bottom Petri dish containing the agar medium was inverted over that containing the sand and flour. Two rapid up-and-down motions served to bring most of the sand and flour against the agar, to which it adhered. The two dishes were then inverted, that containing the flour now being uppermost. The upper dish was tapped sharply, causing the flour adhering to it to fall to the agar. The culture dish was then covered and the process repeated.

Typical results of assaying two flour samples by these three methods are given in Table II. The same batch of malt-salt agar was used in all cases.

The three methods were compared extensively enough, with several

TABLE II

EFFECT OF METHOD OF SAMPLING ON THE NUMBER OF MOLD COLONIES OBTAINED FROM TWO FLOUR STREAMS

Method of sampling	Mold colonies per gram of flour	
	Sample 1	Sample 2
Liquid dilution		
Suspended in medium	1860	5920
On surface of medium	1780	3020
Dry, on surface	1080	4300

flour samples and different media, to demonstrate rather conclusively that a higher and more consistent mold count could be obtained by suspending a liquid dilution in malt-salt medium. If media were used in which bacteria grew moderately well, the dry method yielded higher and more consistent mold counts than either of the other methods.

Comparison of Different Methods of Determining Number of Mold Colonies on Culture Plates. In the methods described in the literature, the number of mold colonies that grew in the culture dishes apparently was determined by visual examination of the dishes without magnification of any kind. In the present tests the mold colonies obtained from more than 10 different flour samples cultured on several different media with the three different methods of dilution were counted with the naked eye and with a binocular microscope. The magnification was approximately X10. Typical results of the two methods of examining plates are given in Table III.

TABLE III

MOLD COUNT AS DETERMINED BY EXAMINING PLATES WITH THE NAKED EYE AND WITH THE AID OF A BINOCULAR MICROSCOPE [1]

Flour stream	Molds per gram [1]— examined by		Percentage increase [2]
	Naked eye	Binocular	
First break	1700	1800	6
First clear	2140	3720	74
Fine-1-tail	3400	5220	54
Suction	4240	5860	30
Coarse-2-tail	4125	7350	78

[1] Each figure is an average of five plates.

[2] Approximate percentage increase of binocular count over naked eye count.

The difference between the two methods of counting was less on those flour streams that contained a small number of colonies of a relatively few species of molds. Plates containing a total of 50 to 100 colonies of several different species of molds could be counted accurately only with some sort of magnification. Many of the colonies of various molds grow slowly when submerged in the agar, and are invisible to the naked eye even when the cultures are 6 or 7 days old. At this age, some of the faster-growing surface colonies of *Aspergillus glaucus* and *A. flavus* began to obscure the small, submerged colonies, and the total as counted by either method began to decrease. There was a high agreement among replicate plates of a given flour sample with each method of counting, but the fact that more colonies were found when a binocular was used, in addition to the previously indicated fact that the composition of the medium affects the total count, shows that consistency alone was not a good criterion of accuracy.

Extreme Variation in Mold Count When Using Different Media and Different Methods of Examining Plates. One sample of flour cultured in the penicillin assay medium and read with the naked eye yielded 160 molds per gram; when cultured by the same method in malt-salt medium and read with the aid of a binocular a count of 1860 molds per gram was obtained. A second flour similarly assayed yielded 960 molds per gram when cultured in the penicillin assay medium and read with the naked eye, and 5920 per gram in malt-salt medium, read with a binocular.

Detailed Description of the Present Method

In view of the fact that several modifications of the methods described in the literature have been devised, it seems desirable to summarize the method of mold assay used in our laboratory.

REAGENTS:

Sodium chloride, C. P.
Malt extract, various commercial brands are suitable.
Agar-agar, crude shredded is suitable, but better grades may be used if desired.
Distilled water
Quartz sand

APPARATUS:

For each flour sample to be assayed the following are required:
Five 90-mm Petri dishes
Two 3-oz screw capped medicine bottles.
Two test tubes approximately 10 mm diameter and 6 cm long, calibrated to deliver 5 ml.
One 1-ml pipette. These are made from pyrex tubing approximately 4 mm inside diameter, drawn out at one end to an opening of 1.5 mm and calibrated to deliver 1 ml.
One rubber dropping bottle bulb to use with the pipette.
Torsion balance sensitive to 0.01 g.

PROCEDURE:

Preparation of medium. The medium is prepared in lots of 500 ml, sufficient to assay five flour samples. Boil 10 g of agar and 10 g malt extract in 350 ml of distilled water until agar is melted. Make up to 350 ml with distilled water. Dissolve 37 g sodium chloride in 150 ml distilled water. Place agar solution in a 16-oz screw-capped medicine bottle, the sodium chloride solution in an 8-oz medicine bottle. Autoclave 20 minutes at 15 lb pressure. Add the sodium chloride solution to the agar and cool to 45°C before pouring. In practice, enough bottles of agar and sodium chloride solution are made up to last several weeks; the agar is melted in the autoclave as needed and the cold sterile solution of sodium chloride added to it.

Preparation of the flour. Weigh out 5 g of flour on a piece of sterile paper on the pan of a torsion balance sensitive to 0.01 g. Place in a 3-oz screw-capped medicine bottle containing 10 g sterile quartz sand. Using a large calibrated test tube, add 47 ml of sterile 0.5% solution of sodium chloride. Forty-seven milliliters are used because it has been found that 5 g of flour suspended in 47 ml of water make a total volume of 50 ml. Place screw cap on the bottle and shake bottle vigorously for 2 minutes. While the liquid is still agitated, and before any of the flour has settled, measure 5 ml into a second 3-oz bottle. This is done by means of a small test tube calibrated to deliver 5 ml. Add 45 ml of sterile 0.5% sodium chloride solution by means of a test tube calibrated to deliver 45 ml. Shake the bottle. While the liquid is still turbulent, remove 1 ml of the suspension by means of the pipette equipped with a rubber bulb, and immediately dispense into a sterile Petri dish. Shake the bottle again and dispense another 1-ml aliquot into another Petri dish. Usually five replicate plates are made of each sample. Cool agar to 45°C. Pour the cooled agar into the dishes, swirl each dish gently to suspend the flour uniformly, and place on a level table. When agar is hard, stack the plates on a shelf and allow to incubate for 5 to 7 days at room temperature (20°–25°C).

The calibrated test tubes and the improvised 1-ml pipette sacrifice a small amount of accuracy in measurement. This sacrifice is more than compensated for by increased speed of sampling, and in time saved in washing glassware. Five flours, with five replicate plates of each, can be sampled in two hours, including the washing of glassware. In our experience these measuring devices have proved equally as accurate as calibrated pipettes, so far as final mold count is concerned, as well as economical of time and labor.

Counting the colonies on the plates. Draw six or seven parallel lines on the bottom of each Petri dish with a wax pencil. Remove the cover and place the plate on the stage of a binocular microscope equipped with a mirror and an aperture in the stage through which the light can be directed. A desk lamp furnishes satisfactory illumination. With a magnification of x10 to x25, colonies throughout the entire depth of agar can be seen. The lines on the bottom of the plate serve as guides in moving the plate back and forth.

Use of controls. Samples of sterile flour are assayed from time to time as a check on the number of mold colonies that may enter from the air or other sources. The flour is sterilized by heating it slowly to 180°C in a gas oven, stirring it occasionally to prevent caking, after which it is left at 180°C for several hours. This sterile flour is assayed in the same way as are other flour samples. Replicate dilutions of 1:100 of this sterile flour seldom average more than 0.5 colony per plate.

Discussion

The present studies indicate that the number of mold colonies obtained from any given flour sample is influenced by the method of making the dilutions, the medium on which the flour is cultured, and the method of reading colonies in the culture dishes. While the malt-salt medium was superior to the other media tested, this does not imply that it will be superior in all cases. If flour streams whose mold flora is unknown are to be extensively sampled, a number of different media probably should be tested.

The assumption is made that the approximately 4-oz sample taken from each stream is representative of that stream. Results of the dry assay method suggest that the molds are uniformly distributed in the flour. The mold count of individual plates among five replicates of a given flour sample by this method seldom varied more than ±5% from the average of the five. This relatively close agreement between replicate 10-mg aliquots of the same flour sample has been the rule, not the exception. This was true not only of the total number of mold colonies, but also of the relative numbers of each of the principal kinds of molds encountered in each sample. Evidently the molds were distributed with surprising uniformity in the samples taken from different streams. Further information on this will be presented in a later paper.

One advantage of examining the culture plates with a binocular microscope is that it permits one familiar with the molds to identify most of them at the time of counting. In some flour streams more than 10 species of molds, belonging to more than five genera, have been encountered, so that merely counting the total number of colonies does not furnish a complete picture of the nature of contamination.

None of the methods of mold assay now available distinguish between mold spores and fragments of mycelium. Nor do they permit one to estimate the number of nonviable spores that may be present. These deficiencies in the method do not seem important, but nevertheless they must be kept in mind. Information on these factors would be desirable in any interpretation of the role and significance of molds in flour.

Summary

Malt-salt medium was superior to the other five media tested, for determining the number of molds in flour. No medium was found on which both molds and bacteria could be assayed together.

The use of a binocular microscope increases the number of mold colonies found on agar plates when compared with the number found without the use of magnification. This is especially true when numerous colonies of several different species or genera of molds are present.

A higher mold count is obtained from a given sample of flour, using malt-salt medium, if the flour is suspended in sterile saline solution and this suspended in the agar, than if it is similarly suspended and cultured on the surface of the agar, or if it is spread dry on the surface of the agar. With other media, a higher count may be obtained by using the dry method.

Acknowledgments

The writer wishes to express his indebtedness to Dr. W. F. Geddes, at whose suggestion this work was undertaken, to Mr. R. M. Finch and Mr. Walter Tholstrup of the Wallace and Tiernan Company, and to the personnel of several mills whose interest and aid have facilitated the work. Thanks are due Dorothy R. Gordon for assistance in various laboratory phases of the work.

Literature Cited

Barton-Wright, E. C.
1938 Studies on the storage of wheaten flour. III. Changes in the flora and the fats and the influence of these changes on gluten character. Cereal Chem. **15**: 521–541.
Kent-Jones, D. W., and Amos, A. J.
1940 Preliminary studies in the bacteriology of wheat and flour. Analyst **55**: 248–267.
Schmidt, W. H., and Moyer, A. J.
1944 Penicillin. I. Methods of assay. J. Bact. **47**: 199–208.
Smith, N. R., and Humfeld, H.
1930 Effect of rye and vetch green manures on the microflora, nitrates, and hydrogen-ion concentration of two acid and neutralized soils. J. Agr. Research **41**: 97–123.

RELATIONSHIP OF ZEIN TO THE TOTAL PROTEIN IN CORN [1]

D. W. HANSEN,[2] B. BRIMHALL,[3] and G. F. SPRAGUE [4]

(Received for publication December 12, 1945)

The nutritional quality of corn germ protein has been recognized as approximating that of animal proteins, both from feeding experiments and comparative amino acid analyses (Block and Bolling, 1944; Mitchell and Beadles, 1944). Osborne and Mendel (1914, 1914a) demonstrated that the alcohol-soluble protein of corn, or zein, was nutritionally incomplete since animals rapidly lost weight on a diet containing zein as the only source of protein. Normal growth was restored by the addition of lysine and tryptophane to the diet. Zein was also demonstrated to be absent from corn germ although it constitutes the major protein of the endosperm. The rest of the endosperm protein, largely glutelin, was shown to contain the amino acids which zein lacks.

The available information regarding the distribution of lysine and tryptophane in different corn proteins is summarized in Table I. Within the limitations of present knowledge it would appear that the

---

[1] Contribution from the Plant Chemistry and Farm Crops Subsections, Iowa Agricultural Experiment Station, Ames, Iowa, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture, Journal Paper No. J-1327 of the Iowa Agricultural Experiment Station. Project 616. Supported in part by a grant from the Corn Industries Research Foundation.

[2] A. E. Staley Manufacturing Company, Decatur, Illinois.

[3] Research Associate, Plant Chemistry Subsection, Iowa Agricultural Experiment Station.

[4] Senior Agronomist, Division of Cereal Crops and Diseases, Collaborator Farm Crops Subsection, Iowa Agricultural Experiment Station.

TABLE I

LYSINE AND TRYPTOPHANE CONTENTS OF VARIOUS CORN PROTEINS

Protein	Lysine	Tryptophane	Reference
	%	%	
Germ protein	5.8	1.3	(Block and Bolling, 1944)
Endosperm protein	1.1	0.6	(Block and Bolling, 1944)
Zein	0.0	0.0	(Osborne and Mendel, 1914)
		0.0	(May and Rose, 1922)
Glutelin	2.93	Strong positive color test	(Osborne and Mendel, 1914)
α-Glutelin	6.71	—	(Jones and Csonka, 1928)
Gluten	—	1.08	(May and Rose, 1922)

determination of zein content in a series of corn samples might provide an index of their relative feeding quality, on the assumption that the higher the zein content of corn protein the lower its feeding value. This analysis is much more easily carried out than are amino acid assays, and, in fact, consists in estimating the amount of protein with zero lysine and tryptophane content rather than directly determining these amino acids in the mixed proteins of corn.

The zein content of corn protein varies widely, values all the way from 28% to 60% having been reported (Showalter and Carr, 1922). The variation in values is due to several factors, among which are:

(*1*) *Method of Analysis.* The concentration of aqueous alcohol used by different investigators to extract the zein has varied from 70 to 95%. Recently it has been shown (Nagy, Weidlein, and Hixon, 1941) that addition of 0.5% sodium acetate as a buffer improves extraction of zein by 85% ethanol. These authors point out that the separation of different corn proteins by solvent extraction methods has proved far from satisfactory as a quantitative procedure, since large variations in the apparent percentages of the different fractions are caused by slight alterations in the procedure (fineness of grinding, length and temperature of extraction, presence of salts, etc.). Nevertheless they consider the alcohol-soluble fraction to be the most well-defined of these and least affected by slight variations in experimental techniques.

(*2*) *Maturity of Corn.* Zeleny (1935) found that zein was nearly absent in very immature corn but was synthesized rapidly as the corn approached maturity. The rapid increase in the ratio of zein to total nitrogen was almost exactly paralleled by the decrease in water-soluble nonprotein nitrogen.

(*3*) *Protein Content of the Corn.* Showalter and Carr (1922) were the first to point out that the proportion of zein to total protein is greater when the total nitrogen content of the corn is higher. An increase in zein was accompanied by a corresponding decrease in the glutelin (dilute alkali-soluble) fraction. Using 90% alcohol as the extracting agent, they reported 8.1% zein in corn containing 15.7% protein and 2.2% zein in corn with 8.0% protein.

The present paper reports a more extensive study of the quantitative relationship between zein and total protein in whole corn and in corn endosperm.

Materials and Methods

The corn samples represented a varied assortment of inbred lines, single crosses, commercial hybrids; white and yellow corn; floury, dent, and waxy; high- and low-oil. The sweet corns included several waxy-sweet types; pseudo-starchy as well as extremely sweet samples; varieties such as Golden Bantam, Country Gentleman, and Evergreen; early and late maturing.

For alcohol-soluble protein, either the AOAC method (Association of Official Agricultural Chemists, 1940) or the following modification was used: Two grams of ground sample was weighed into a 250-ml centrifuge bottle, 50 ml of 70% ethanol added, and the stoppered bottle rotated continuously for 24 hours at room temperature. The contents were then centrifuged and 20-ml aliquots of the supernatant fluid taken for nitrogen analysis using the Kjeldahl method. On several samples of corn meal, 70% ethanol was found to extract the same amount of protein as did 85% ethanol buffered with 0.5% sodium acetate.

For separation of the endosperm, corn kernels covered with water were placed in a 50°C oven for 2 to 3 hours, after which the pericarp could readily be peeled off and the germ removed with a scalpel. After drying, the endosperm was ground to 40 mesh in a small Wiley mill. Total protein was determined on half-gram samples. All data were calculated to a moisture-free basis.

Results

The senior author illustrated that a highly significant correlation exists between zein and total protein content. Eighteen different corn samples covering the range from 6.3 to 19.7% protein were used. When 70% alcohol-soluble protein (Y) is plotted against total protein (X) the points fall along the regression line shown in Figure 1.

Data for 36 samples of endosperm from flour and dent corns are plotted in a similar manner in Figure 2 (solid line with circles) bearing out the linear relationship found for whole corn.

The amount of alcohol-soluble protein in sweet corn endosperm was found to be less than that in dent corn, averaging 1% less in the

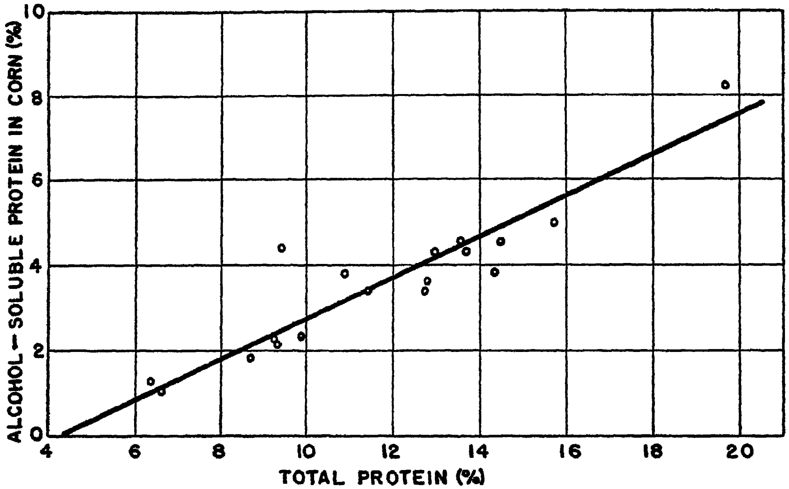


Fig. 1. Relationship between alcohol-soluble and total protein in dent corn.
$r = 0.923^{14}$ $Y = 0.478X - 2.0$

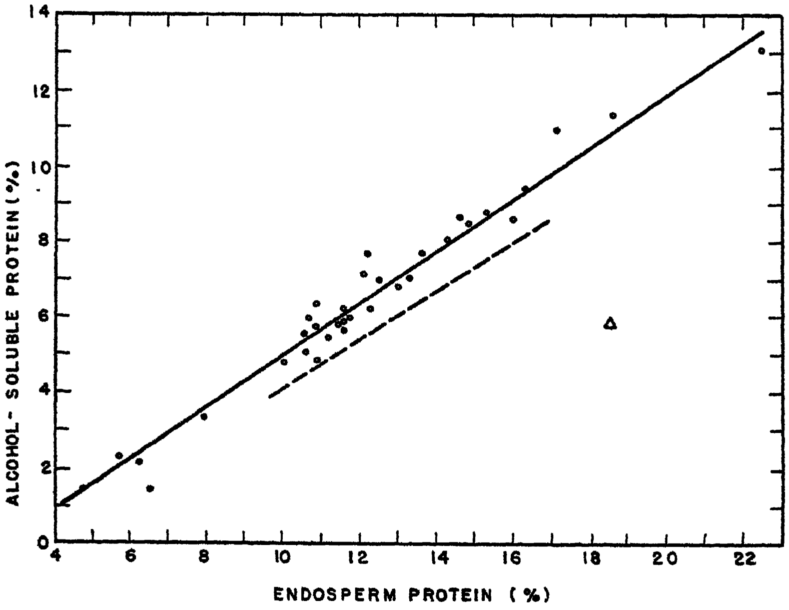


Fig. 2. Relationship between alcohol-soluble and total protein in corn endosperm.

Dent corn ——— Sweet corn - - - - - Brittle corn △

$\begin{cases} r = 0.984^{**} \\ Y = 0.692X - 1.87 \end{cases}$ $\begin{cases} r = 0.778^{**} \\ Y = 0.657X - 2.44 \end{cases}$

range measured (dotted line, Fig. 2). Thus at the 13% protein level, dent had 7.1% zein while sweet had 6.1%. Samples of sweet corn with extremely high or low protein content were not available and the correlation coefficient for the 30 samples analyzed, although highly significant, was lower than for the dent corns. This is not surprising in view of the variation in composition of corn classified as sweet. For example, the samples used varied from 14 to 42% in the amount of water-soluble constituents.

A third type of corn, known as "brittle," was also investigated (Mangelsdorf, 1926). It resembles sweet corn in appearance but is much more shriveled. The endosperm of one sample of brittle corn was found to contain 6.07% alcohol-soluble protein and 18.7% total protein, placing it far to the right of the lines for dent or sweet corn (Fig. 2).

Discussion

Prediction of nutritional values from analytical data is still in an uncertain state of development (Crampton, 1945). Nevertheless it seems logical that the feeding quality of corn protein would be inversely related to its zein content because of the latter's deficiency in the nutritionally essential amino acids, lysine and tryptophane. The value of the data presented in this paper lies in their use for detecting samples which depart widely from the average, in the direction of low zein content. Such samples could then be used in a breeding program with the hope of developing a high-protein, low-zein type of corn.

Mangelsdorf (1926) advanced the hypothesis that the maximum development of corn endosperm is reached in the normal starchy condition, and that the various endosperm types follow the same general growth pattern but may reach quite different end points when growth ceases. Using the weight of mature starchy kernels as a criterion he found that sweet corn reached only 88.5% and brittle corn only 62.5% of equivalent development; in other words, their seed weight when growth ceased corresponded to that of ordinary corn at earlier immature stages. This analogy may be extended to carbohydrate reserves. In dent corn the bulk of the carbohydrates is in the form of starch stored in organized granules. Sweet corn exhibits a less complete synthesis and a considerable part of the carbohydrate fraction is stored as water-soluble dextrins. Brittle corn is typified by an even less complete synthesis, the starch content being low and most of the soluble carbohydrates remaining in the sugar stage.

There are similar indications of incomplete development in the protein of these types of corn. On the basis of Zeleny's (1935) observation that the zein:total-protein ratio increases with advancing maturity, both brittle and sweet corn can be said to have zein:total-protein ratios corresponding to immature dent corn of equal protein content. In each case it appears that protein synthesis follows the same general sequence but that different end points are reached at maturity. For example, if a comparison is made of endosperm tissue containing 6% zein, the graphs show that this constitutes for dent corn 52.7%, for sweet corn 46.8%, and for brittle corn 32.5% of the total protein. The fact that the slopes of the regression lines in Figure 2 do not differ significantly suggests that synthesis follows the same general pattern in dent and sweet corn. This analogy is suggestive but should not be considered as an established fact.

Summary

Alcohol-soluble protein (zein) was determined on samples of corn and of corn endosperm having wide variation in protein content. The data indicate that there is a linear relationship between the content of alcohol-soluble protein and total protein.

Sweet corn endosperm contained an average of 1% less zein than dent corn of equal protein content. "Brittle" endosperm was found to be exceptional in its low zein:total-protein ratio. Apparently the protein as well as the carbohydrate in these types of corn differs from ordinary dent types in the proportion of its constituents.

Acknowledgments

The authors are indebted to Jean and Jane Magee, Willis Guthrie, and Lee Lumpkin for laboratory assistance; and to Dr. E. S. Haber who provided the sweet corn samples.

Literature Cited

Association of Official Agricultural Chemists
1940 Official and Tentative Methods of Analysis (5th ed.), p. 217. Association of Official Agricultural Chemists, Washington, D. C.
Block, R. J., and Bolling, D.
1944 Nutritional opportunities with amino acids. J. Am. Diet. Assoc. 20: 69–76.
Crampton, E. W.
1945 Animal nutrition—a challenge to the food chemist. Trans. Am. Assoc. Cereal Chem. 3: 147–157.
Jones, D. B., and Csonka, F. A.
1928 Studies on glutelins. IV. The glutelins of corn (*Zea mays*). J. Biol. Chem. 78: 289–292.
Mangelsdorf, P. C.
1926 The genetics and morphology of some endosperm characters in maize. Conn. Agr. Expt. Sta. Bull. 279.
May, C. E., and Rose, E. R.
1922 The tryptophane content of some proteins. J. Biol. Chem. 54: 213–216.
Mitchell, H. H., and Beadles, J. R.
1944 Corn germ; a valuable protein food. Science 99: 129-130.

Nagy, D., Weidlein, W., and Hixon, R. M.
1941 Factors affecting the solubility of corn proteins. Cereal Chem. **18**: 514–523.
Osborne, T. B., and Mendel, L. B.
1914 Amino-acids in nutrition and growth. J. Biol. Chem. **17**: 325–349.
1914a Nutritive properties of proteins of the maize kernel. J. Biol. Chem. **18**: 1–16.
Showalter, M. F., and Carr, R. H.
1922 Characteristic proteins in high- and low-protein corn. J. Am. Chem. Soc. **44**: 2019–2023.
Zeleny, L.
1935 The distribution of nitrogen in the seed of *Zea mays* at different stages of maturity. Cereal Chem. **12**: 536–542.

BOOK REVIEWS

Frontiers in Chemistry. IV. Major Instruments and Their Applications to Chemistry. Edited by R. E. Burk and Oliver Grummitt. viii and 151 pp. Interscience Publishers, Inc., New York, N.Y. 1945. Price $3.50.

This fourth volume of the Western Reserve University Frontiers in Chemistry is intended to tell the chemist what information may be obtained from the application of six different types of physical instruments to problems in various fields of chemistry.

Chapter I, "Electron diffraction and the examination of surfaces," by Lester H. Germer, is a reprint of an article by the same author in Jerome Alexander's "Colloid Chemistry," Volume V, 1944. It describes the use of electron diffraction cameras, or of slightly modified electron microscopes, for the study of the structure of surface films. These measurements give data analogous to X-ray diffraction patterns, but their limited penetrating power results in the pattern being produced only by the surface layer. Extremely minute samples are therefore adequate for an analysis.

Chapter II, "The electron microscope and its applications," by L. Marton, summarizes the theory of the increased resolving power and the construction of the electron microscope and gives pictures of some details of the author's machine. Applications to colloids, plastics, rubber, soaps, certain chemical reactions, metal surfaces, and biological material are described in sufficient detail to show the possibilities and limitations of the apparatus in various fields.

Chapter III, "X-ray diffraction and its applications," by Maurice L. Huggins, covers its field in more detail than the other chapters. The nature of X-rays, their production, and the systems used for taking the different types of photographs are described. The use of diffraction photographs for determination of the structure of known compounds and their use in identifying substances is described with many examples and crystal structure diagrams of both inorganic and organic substances.

Chapter IV, "Chemical spectroscopy," by Wallace R. Brode, briefly discusses the nature of light and the units used in measuring its wave length. The emission of spectral lines is illustrated by the energy level diagram of sodium atoms. The use of tables and charts for line identification is explained with examples, and a short description of the properties of the photographic plate is followed by a discussion of qualitative and quantitative analysis for metallic elements by emission spectroscopy.

Chapter V, "Application of absorption spectra to chemical problems," also by Brode, starts with the systems of units both for absorption coefficients and for wave length or frequency that are used in absorption spectroscopy. A particularly useful diagram is included which shows the same data plotted as percent transmission, extinction, (log I_0/I), and as log extinction. The connection between chemical structures and the absorption of light is clearly presented with absorption curves for various chromophores. Effects of conjugation and interference of various groups in organic molecules are illustrated by curves. The resolution of an absorption curve into its component bands is illustrated by $KMnO_4$ and by several organic compounds. This chapter is designed primarily to indicate how the organic chemist may use absorption spectra as an aid in elucidating the structure of a substance, since the purely analytical uses are already widely appreciated.

Chapter VI, "The infrared spectrometer and its application," by R. Bowling Barnes, gives a short history of infrared spectroscopy, describes the relation of infrared to other parts of the electromagnetic spectrum, and shows how infrared absorption spectra of organic molecules are related to the atomic groups present. Descriptions both of a large research model and of a smaller infrared spectrophotometer are given together with comments on the experimental difficulties involved in the design of such instruments. Typical applications to identification of organic molecules, determination of structure, quantitative analysis of several components of mixtures, measurement of reaction rates, and process control are clearly described as being an extension of the more familiar spectroscopic procedures to the infrared region.

Each chapter is contributed by a recognized authority in the special fields, but the book as a whole is so short that none of the chapters can be considered adequate to give a working knowledge of any of the techniques discussed. However, its purpose, to give the average chemist some information as to how the various instruments can be applied to different purposes, is well achieved.

C. S. French
University of Minnesota
Minneapolis, Minnesota

Organic Preparations. By Conrad Weygand. 534 pp. Interscience Publishers, Inc., New York, N. Y. 1945. Price $6.00.

This book is a translation of Part II, "Reaktionen," of Conrad Weygand's *Organisch-chemische Experimentierkunst*, published in Leipzig in 1938. Parts I and III of the German original, which deal with laboratory equipment and the chemical and physical analyses of organic compounds, have been omitted in the translation. The general plan and material in Part II of the German book have been followed quite closely. There is one major difference. The original contains numerous literal quotations of preparations published in *Organic Syntheses;* these have been replaced in the translation by references to *Organic Syntheses* and wherever possible references to single volumes of *Organic Syntheses* have been replaced by references to the Collective Volumes I or II.

The material is arranged by a new system based upon the formation of various linkages of the carbon atom and their fission. The method of organization may best be indicated by the chapter headings:

(1) Formation of Carbon-Hydrogen Bonds
(2) Formation of Carbon-Halogen Bonds
(3) Formation of Carbon-Oxygen Bonds
(4) Cleavage of Carbon-Oxygen Bonds
(5) Formation of Organic Derivatives of Trivalent Nitrogen
(6) Cleavage of Carbon-Nitrogen Bonds
(7) Formation of Carbon-Pentavalent Nitrogen Linkages
(8) Carbon-Divalent Sulfur Bonds
(9) Carbon-Hexavalent Sulfur Bonds
(10) Unsaturated Carbon Bonds
(11) Formation of Carbon-Carbon Bonds
(12) Fission of Carbon-Carbon Bonds
(13) Rearrangements of Carbon Compounds with the Exception of Steric Rearrangements.

Numerous methods for carrying out the transformations are discussed briefly and each type of reaction is characterized and its usefulness illustrated by specific laboratory procedures. This enables one to determine the applicability of a laboratory process and to select the best method for the solution of some particular problem. Many references to the literature make it possible to find further information concerning each method.

This translation makes available the most valuable section of Conrad Weygand's excellent German text. It fills the need for a working laboratory manual which is more advanced than the usual laboratory text books and which is up to date and gives more recent information concerning organic preparatory methods than is found in Houben-Weyl's classic handbook, *Die Methoden der Organischen Chemie.*

Sidney E. Miller
General Mills, Inc.
Research Department
Minneapolis, Minnesota

CEREAL CHEMISTRY

VOL. XXIII JULY, 1946 No. 4

PHOTOMICROGRAPHIC STUDIES OF WHEAT STARCH. I. DEVELOPMENT OF THE STARCH GRANULES [1]

R. M. SANDSTEDT

Department of Agricultural Chemistry, Nebraska Agricultural Experiment Station, Lincoln, Nebraska

(Received for publication March 4 1946)

The starch granule has been the subject of an immense amount of microscopic study beginning with the work of Leeuwenhoeck in 1716 who concluded that the starch grain consisted of an outer insoluble, indigestible coat and an inner nutritive substance which disappeared when the granule was heated in water. Reichert (1913) gave a thorough review of the starch literature including that dealing with the origin and growth of the starch granule. He found the literature quite contradictory: "Very frequently the reports of one observer are not confirmed or are absolutely contradicted by those of others, even when the same method or reagent has been employed. . . ." (The starch granule), "as Poggendorff pointed out in 1836, was up to that time one of the most studied and least understood of all substances." Badenhuizen (1939) indicated that the situation was not much better in 1938. He suggested that if we review our present knowledge of the structure of the starch granule we are struck by the remarkable fact that we have actually arrived at a plane of knowledge equal to that of one hundred years ago.

According to Reichert (1913), Fritzsche (who apparently was the first to study the mechanism of the formation of the starch granule) concluded that all granules were formed by the deposition of the outer layers upon the inner. This "apposition" theory of the growth of the starch granule was accepted by other investigators until Walpers in 1851 proposed the view that growth was by "intussusception"; i.e., growth from the outside inward or by deposition within and between material already laid down. These opposing views led to considerable controversy which has not been entirely settled at the present time

---

[1] Published with the approval of the Director as paper No. 394, Journal Series, Nebraska Agricultural Experiment Station

(Wieler, 1938) although it seems that the theory of growth by external apposition is quite generally accepted.

Investigations of the development of the wheat kernel have been extensive (Brenchley, 1909; Brenchley and Hall, 1909; Thatcher, 1913 and 1915; Eckerson, 1917; Jensen, 1918; Percival, 1921; Gordon, 1922; Miller, 1939; and many others). Since there is considerable similarity in the development of the grain of the different cereals, such investigations as those of Harlan (1920), Mottier (1921), Weatherwax (1930), Lampe (1931), and Randolph (1936) also throw considerable light on the development to be expected in wheat. However, none of this work was designed specifically to show the details of the growth and development of the starch granules.

Teller (1938) published photomicrographs of immature wheat and barley starch which supported his hypothesis that the amylases of grains and tubers were active in the formation of the starch granule, i.e., that growth of the starch granule was the antithesis of starch degradation by the enzymes. Bice, MacMasters, and Hilbert (1945) pointed out some of the differences between mature and immature wheat starch. Evans (1941) made a study of the development of corn starch and showed photomicrographs of granules at different stages of growth.

Wheat starch is composed of granules varying in diameter from 2 μ to 50 μ. It has been hypothesized that the ratio of small to large granules in a wheat has an effect on the baking properties of its flour (Buchanan and Naudain, 1923; and Stamberg, 1939), although Grewe and Bailey (1927) could find no relationship between baking properties and size of starch granules.

MacMasters and Hilbert (1944) state that in the preparation commercial wheat starch much of the small granule starch is lost from the better grades of starch and is recovered in the tailings. Consequently, in the manufacture of wheat starch the quantity of small granule starch is of industrial importance and a study of the origin and development of this starch may yield information of value to the wheat industry. It is the purpose of the present paper to present our observations concerning the development of wheat starch.

Materials and Methods

Selection of Kernels of Wheat of Approximate Known Age. For this study the procedure of making daily harvests of wheat kernels (Brenchley, 1909) was used, starting with the ovary before fertilization and continuing to the maturity of the grain. In order to have material of approximately known age, heads of hard winter wheat were dated at the first signs of pollination (extrusion of the first anthers); one kernel was harvested from each of several of these heads each day. This procedure does not insure the exact age of the kernels since there may be considerable difference in the age of kernels on the same head (Brenchley, 1909); however, by harvesting kernels from several heads each day those obviously out of line in development could be discarded. Field-grown wheat was used during the first season of this study (1941), whereas both field and greenhouse samples were used during the succeeding seasons.[2]

Starch Preparations. Preliminary preparations consisted of starch squeezed directly from a kernel onto a microscope slide. In later studies the pericarp (plus the epidermis of the nucellus) and endosperm were separated permitting starch development to be followed in both the endosperm and pericarp. In those cases requiring washed starch, the starch was pressed from the tissues into water and washed by repeated suspension, settling or centrifuging, and decantation.

Cross sections of wheat kernels were cut on a freezing microtome using ethyl chloride as the freezing agent. Sections were cut both from fresh material and from material which had been fixed in formalin acetic alcohol.[3] The sections used for photomicrographic illustration were all fixed in formalin acetic alcohol.[4]

The photomicrographs were made under the direction of R. F. Morgan in the laboratories of the Department of Visual Education, using Bausch and Lomb GBVP photomicrographic equipment. A B & L mechanical-feed arc lamp and B & L polaroid disc polarizer and cap analyzer were used for studies involving birefringence. Such polaroid equipment gives results comparable to those obtained with a polarizing microscope (Young, 1944).

Movement of starch granules due to convection currents and to drying of the slides was prevented by sealing the sample under the cover glass. A narrow band of vaseline was placed around the edge, on one face, of the cover glass. After the sample was prepared on the slide, the cover glass was gently pressed into place using care to have the vaseline make a complete seal (McNair, 1930). Starch granules in samples thus sealed under the cover glass may be kept for weeks with little perceptible movement. No undue pressure should be applied to the cover glass as starch granules are quite easily damaged by pressure (Jones, 1940).

In the early stages of growth the starch granules were so small that they were subject to too much Brownian movement for observation or for photographing while in the original suspension. It was found that Brownian movement could be largely prevented, with no perceptible change in the character of the starch, by allowing the starch suspension to dry on the slide. On carefully adding water (or the particular solution desired) the granules were left lightly sealed to the glass; this permitted observation and photographing of granules less than 1 μ in diameter.

---

[2] Wheat used for these studies was grown primarily for other purposes by the Agronomy Department of the Nebraska Experiment Station.

[3] We are indebted to Dr. E. R. Walker of the Botany Department for her suggestions and aid in making these sections.

[4] Formalin acetic alcohol. Mixture of 7 *ml* acetic acid, 5 *ml* formalin, and 88 *ml* 50% ethanol.

Staining. Most of the photomicrographs were made of unstained granules and of unstained sections of kernel tissue. However, in some cases it was necessary to use iodine as a stain in order to distinguish minute starch granules from other organized material in the cells of the kernel. The iodine concentration to be used was determined for each particular set of conditions by preliminary tests on similar material. Best results were obtained by using relatively large volumes of the minimum concentration of iodine which would stain the granules of the preparation under consideration.

Precise differential staining between different starch granules, or between parts of an individual granule, necessitated the use of relatively large volumes of exceedingly dilute iodine (0.0007 *N*). Inadequate volume of solution gives apparent differentials which may be quite misleading; this is due to the adsorption of iodine by the first granules contacted, thus leaving a lower concentration for succeeding granules. Too high concentrations of iodine stain all granules and all parts of granules so deeply that no distinctions may be made. Generally it is necessary to make preliminary observations of the effect of iodine concentration on the particular starchy material under study. Similar to the difference observed by Jones (1940) between damaged and undamaged granules, immature granules stain more readily and with lower concentrations of iodine than do mature granules.

Results and Discussion

Figure 1, showing the changes in the external appearance of the wheat kernel during its growth and development, is given as a reference for correlating the development of the starch with that of the kernel. Figure 1 *A* represents the unfertilized ovary, with *B*, *C*, *D*, *E*, *F*, and *G* following at two-day intervals; *H* is the mature dried kernel harvested about four weeks after pollination. At a stage corresponding to *F* the kernel has reached its maximum length but has attained less than half of its final dry weight (Brenchley and Hall, 1909; Percival, 1921; Miller, 1939).

Early Starch Development in the Ovary and Growing Kernel. Minute spherical starch granules, varying in diameter from about 1 μ to 5 μ, occur even in the unfertilized ovary (Fig. 2 *A*). That these are in reality starch granules may be shown by their characteristic appearance under polarized light, although birefringence of the smallest granules is questionable (Fig. 2 *B*), and by their staining with iodine (Fig. 2 *C*). Badenhuizen (1939) and Lampe (1931) also observed young starch granules of narcissus and of corn which showed no birefringence Bice, MacMasters, and Hilbert (1945) account for

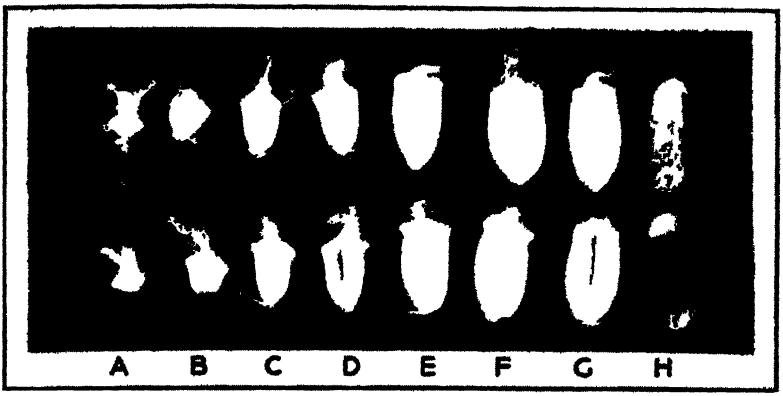


Fig 1 Wheat kernels showing change in size and form during development (×2 5).
A Unfertilized ovary
B–G Kernels approximately representative of development to be expected at 2 day intervals.
H Mature dry kernel

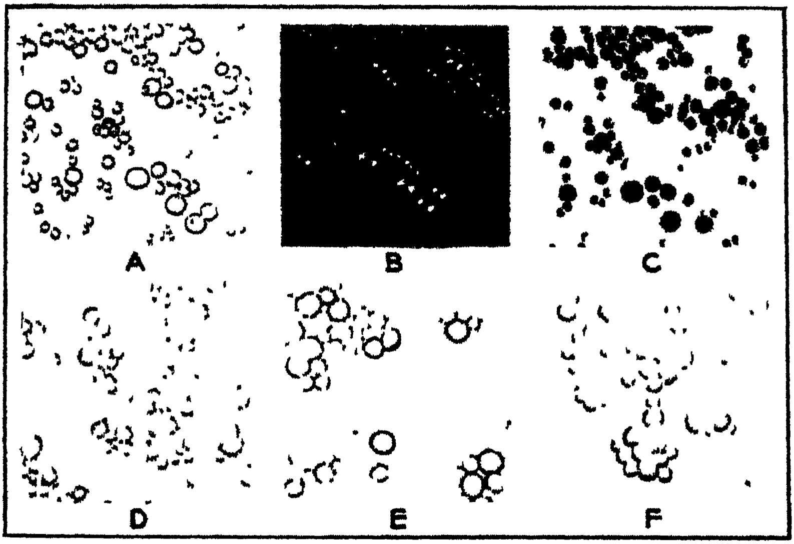


Fig 2. Early starch development of the wheat kernel (×538).
A Starch of the unfertilized ovary
B Field A between crossed polaroids.
C Field A stained with iodine
D Starch from the entire kernel harvested the 2nd day after pollination.
E. Harvested 3rd day
F. Harvested 4th day.

these failures to observe birefringence by the supposition that the illumination used was inadequate to show birefringence in small granules, yet their own illustration, as published, does not show birefringence in the smaller granules. This indicates less distinct birefringence in these granules with possibly no birefringence in the smallest. The B & L carbon arc light used in the present research gave adequate lighting

The starch granule grows with the development of the kernel, as is illustrated in Figure 2 *D*, *E*, and *F* representing the starch squeezed from the whole wheat kernel on the 2nd, 3rd, and 4th days after pollination

Except for the increase in size, there was no readily apparent change in the character of the starch during the first four days; however, at about the 4th day after fertilization corresponding to approximately stage *C* (Fig. 1) in the growth of the kernel, another type of starch was observed. The indistinct (due to small size and transparency) oblong or bean-shaped granules shown in Figure 3 *A* were characteristic of this second type of starch as it was first observed.

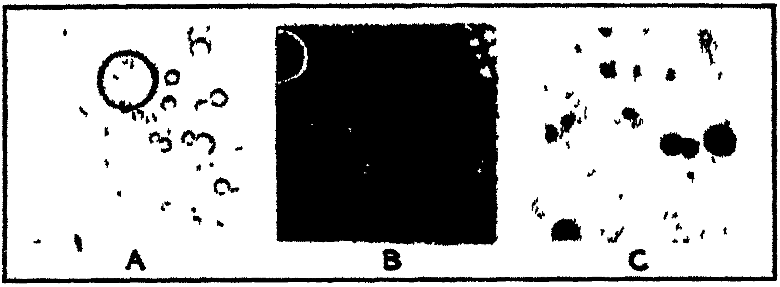


Fig 3 First appearance of endosperm starch in the kernel (×564).
A. Harvested 4 days after pollination circle encloses a group of endosperm granules
B. Between crossed polaroids Circle encloses the same group of initial endosperm granules in *A* and *B* The field was shifted to bring two granules of normal birefringence into view.
C Stained with iodine—original field lost in staining

Figure 3 *B*, showing this starch under polarized light, indicates that any birefringence which it may have had was so slight as to be questionable; this may be due merely to the concentration of starch being too low [5] or to there being too little crystalline starch present at this stage to produce perceptible birefringence. As before noted, Badenhuizen (1939) and Lampe (1931) also observed young starch granules which showed no birefringence. Even though this starch does not show birefringence, it stains with iodine (Fig. 3 *C*). The original field of

starch shown in Figure 3 *A* and *B* was lost in the manipulation necessary for staining but both types of starch are evident in the field shown.

---

[5] That the quantity of starch (thickness of the layer of starch) through which the polarized light passes may have considerable effect on the apparent birefringence of starch granules may be noted if the birefringence of overlapping immature granules is observed, the overlapping region shows more distinct birefringence than those regions not overlapping

The second type of starch develops rapidly: in one day's time the starch of the wheat kernel changes from one predominately spherical in nature to one of a mixture of various shapes. Figure 4 shows

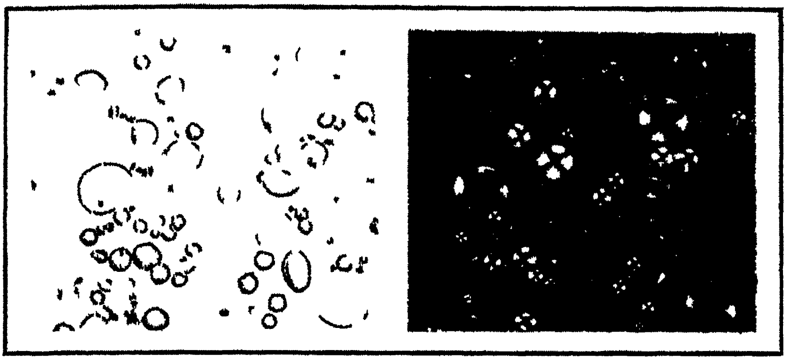


Fig. 4. Starch from a kernel 5 days after pollination (×538). Right—between crossed polaroids.

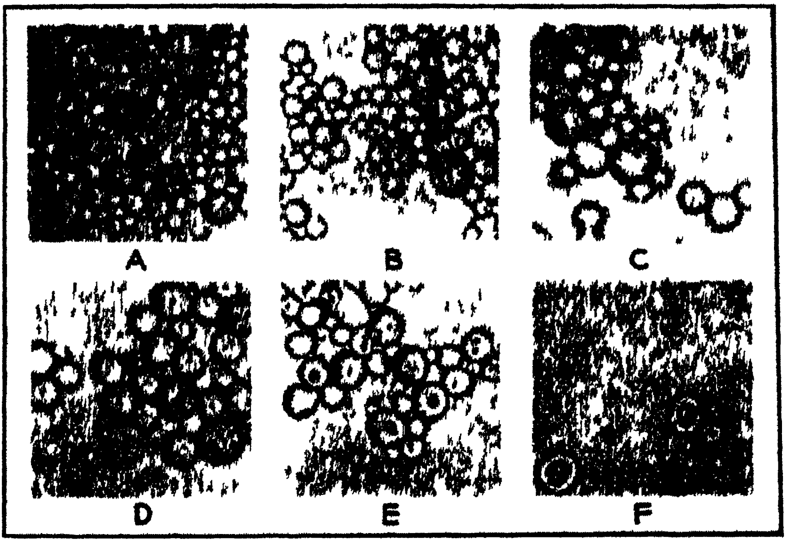


Fig. 5. Development of pericarp starch granules (continued from Figure 2) (×564).

A. 5th day—evidence of enzyme action at the hilum of the larger granules and some pitting of the granule surface.
B. 7th day.
C. 9th day.
D. 12th day—marked pitting of the surface of granules.
E. 14th day.
F. 16th day—the few granules left were largely digested.

starch as it appeared on the 5th day after pollination. At this stage the original spherical type starch tentatively may be distinguished from the new by its greater density, more definite birefringence, and different type of hilum.

Brenchley (1909) stated that "in all the younger stages there is an accumulation of starch in the pericarp." Jensen (1918) also stated that starch makes its appearance in the pericarp tissue long before any vestige of it is present in the endosperm. According to the literature (Brenchley, 1909; Miller, 1939; Percival, 1921; and others) starch storage in the *endosperm* starts after the tissue of the kernel has been completely formed; i.e., 10 to 14 days after fertilization of the flower (or stage *F* of Fig. 1). This would indicate that both types of starch granule originated in the pericarp. However, when the pericarp and endosperm from kernels at various stages of development were separated and the starch from each was studied, it was found that the starch from the pericarp was all of the spherical type and that the endosperm contained starch as early as the 4th day after pollination.

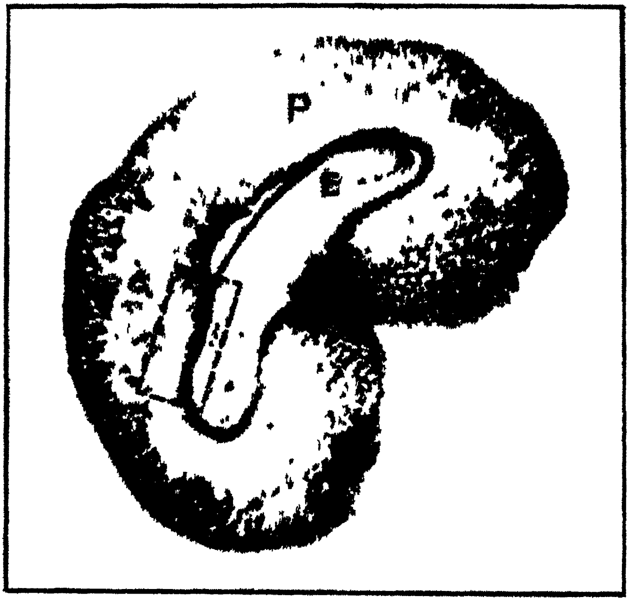


Fig 6 Cross section of wheat kernel 3 days after pollination (×30)

P. Pericarp.
L. Free nucleate endosperm.
The dark border around the endosperm is composed of the ovular integuments and the nucellar wall (See details in Figure 7)
Dotted lines enclose area approximately corresponding to that in Figure 7.

The development of the pericarp starch from the 5th to the 14th day is shown in Figure 5. (This is a continuation of development shown in Figure 2.) As stated by Brenchley (1909), the pericarp starch gradually decreases in quantity with time. Evidence of enzymatic digestion of the pericarp starch granules began to be noticeable by about the 4th or 5th day (Figs. 2 *F* and 5 *A*). In many of the granules digestion appeared to start at the hilum with the innermost portions of the granule disintegrating before the outer layers (Fig. 5 *C* and *F*) indicating that the first starch deposited (in the interior of the granule) was less resistant to amylase action than the outer and younger layers. It should be emphasized, however, that the enzyme

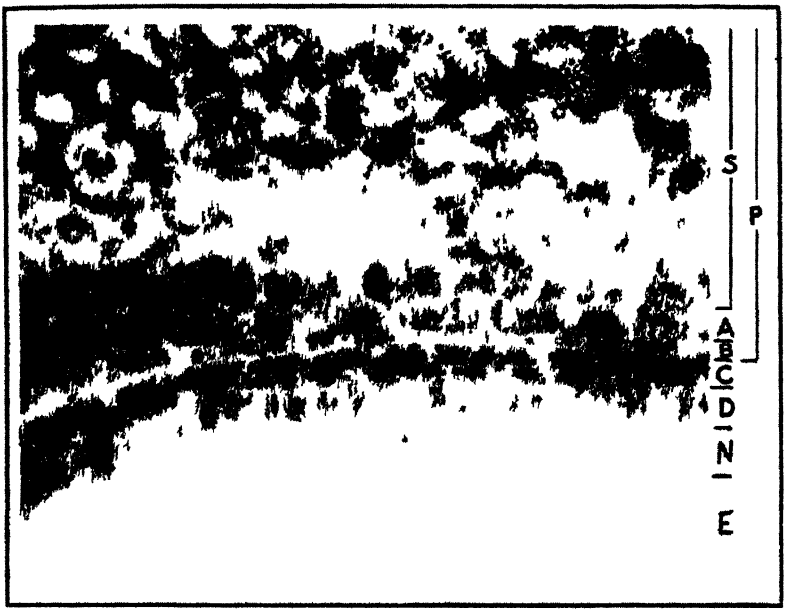


Fig. 7. Portion of a cross section of a wheat kernel 3 days after pollination (×164).
P Pericarp with starch cells (S), cross layer (A), and inner epidermis (*B*).
C Integuments of the ovule.
D Epidermis of the nucellus.
E Endosperm space from which the free nuclei were lost in cutting the section.
N Remains of the thin walled nucellar layer.

had digested channels through the outer layers of the granule before it had access to the interior; in some cases the surface became noticeably pitted before the disintegration of the interior (Fig. 5 *D* and *E*). Apparently the differences in resistance to amylase action between the inner and outer layers varied from granule to granule. By the 16th day, about two to four days after the kernel had reached maximum length, the pericarp starch had practically disappeared (Fig. 5 *F*).

Early Development of the Endosperm. To determine the time and place of origin of the two types of starch which were obtained from the whole kernel on the 5th day of its development (Fig. 4), kernels of wheat representing the early growing period were sectioned. Figure 6, a cross section of a wheat kernel three days after pollination, shows the relation of the pericarp to endosperm at a stage of kernel development when only pericarp starch was present. The greater portion of the kernel consisted of pericarp which was largely composed of starch cells. The endosperm was small and contained no starch. Weatherwax (1930) describes the endosperm at this stage as a "multinucleate cell" which has developed from the definitive nucleus by repeated division; i.e., the endosperm consisted of a small mass of protoplasm containing many nuclei with no cell walls separating the nuclei.

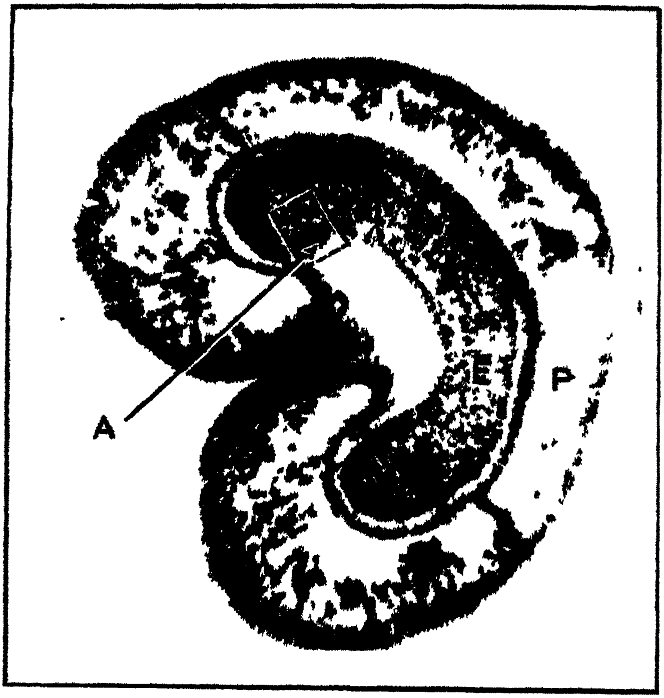


Fig 8 Cross section of a wheat kernel 4 days after flowering (×30)
P. Pericarp
E. Endosperm.
A. Area corresponding approximately to that shown in Figure 9.

The division of the endosperm into cells, i.e., the formation of cell walls between the nuclei of the endosperm, begins at the periphery of the "multinucleate cell" (Brenchley, 1909, and Percival, 1921). Figure 7 shows a portion of a section, thinner than that shown in Figure 6 and with higher magnification. The free nucleate endosperm was lost in cutting the thin section.

Figure 8 shows the relative development of the pericarp and endosperm in a cross section of a wheat kernel harvested four days after pollination (corresponding to stage *C* of Figure 1). A section of the endosperm before and after staining with iodine (corresponding to the area designated by *A* in Figure 8) is shown in Figure 9. The division of the endosperm into cells was exceedingly rapid, in this case seemingly it was complete in about one day's time (Brenchley, 1909, estimated the time as approximately two days).

Endosperm Starch. Even at this early stage, apparently as soon as the division of the endosperm into cells was complete, the deposition of starch had begun in the inner endosperm cells. The starch granules as they were just beginning to form were difficult to distinguish from other structures of the cell until stained with iodine. Figure 10 shows a starch cell, stained with iodine, which was isolated from the endosperm at this stage of development. The stained starch granules of this cell are similar to those shown in Figure 3. Apparently endosperm starch starts to develop much earlier than has been supposed (Brenchley, 1909; Miller, 1939; Percival, 1921).

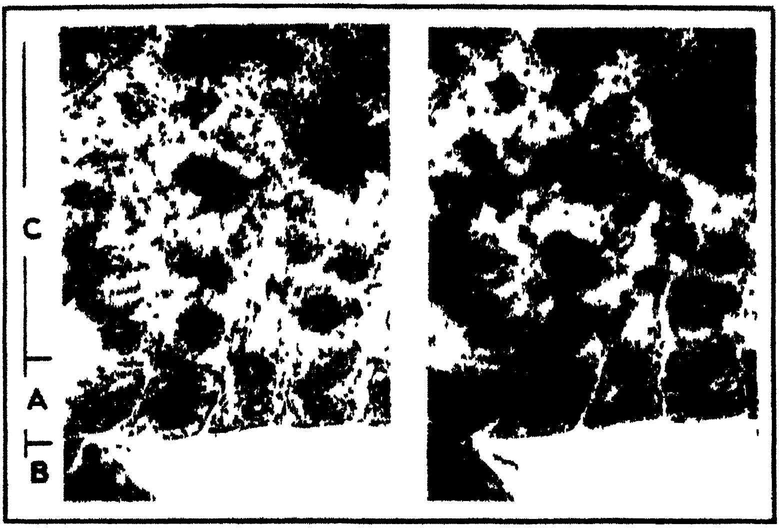


Fig. 9. Section of endosperm 4 days after flowering (×312)
Right—same section stained with iodine
A. Aleurone layer—peripheral layer of endosperm cells
B Epidermis of the nucellus
C. Endosperm with the inner cells showing initial starch granules

The peripheral layer of cells of the endosperm, which was the first to develop cell walls (Brenchley, 1909; Percival, 1921; and Gordon, 1922), did not contain starch (Fig. 9).

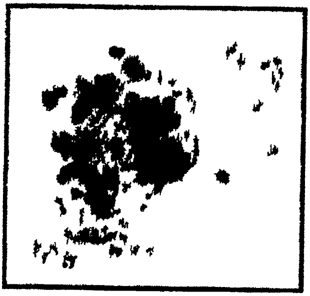


Fig 10 Endosperm starch cell with initial granules stained with iodine (×600

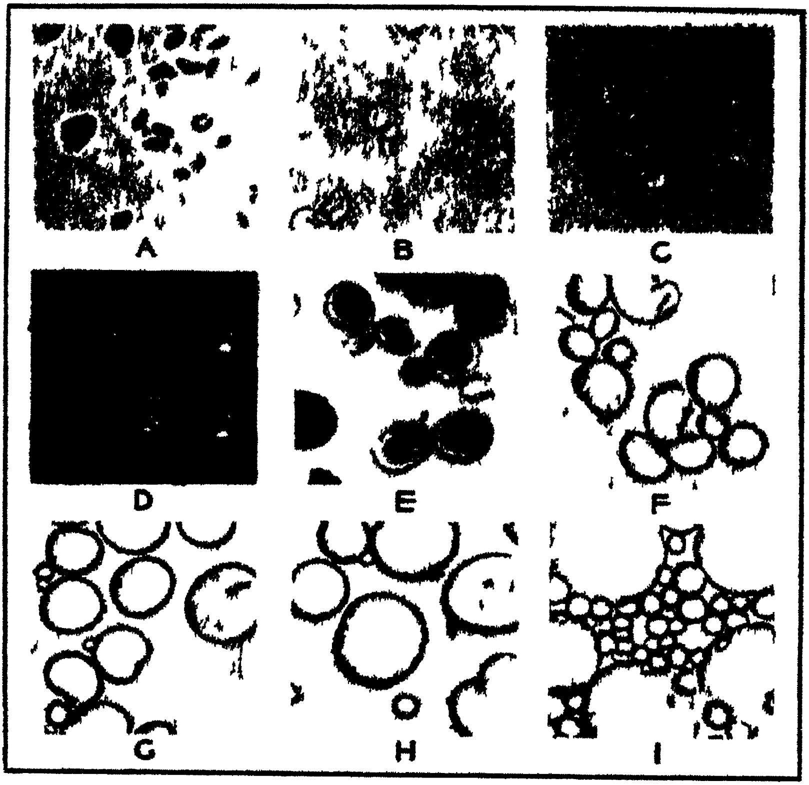


Fig 11 Development of endosperm starch granules (*A* ×1200 *B–I* ×564)
A Initial granules—5th day after pollination Stained with iodine (×1200)
B 7th day
C 9th day
D Same field as *C* between crossed polaroids
E 9th day stained with iodine
F 11th day
G 13th day—first appearance of spherical granules
H 15th day
I Granules from mature dry wheat

The first endosperm starch that could be isolated was quite transparent and did not photograph satisfactorily. Stained with iodine it appeared as shown in Figure 11 *A* (compare with Fig. 10). The development with time is shown in the succeeding photomicrographs of Figure 11. It is interesting to speculate on the method of growth of the granules. Perhaps, judging from the shapes of the granules observed during the early stages of growth, the individual granule develops through a series of forms as indicated by the numbers *1*, *2*, *3*, etc., in Figure 12. Beginning with a sphere which serves as a "nu-

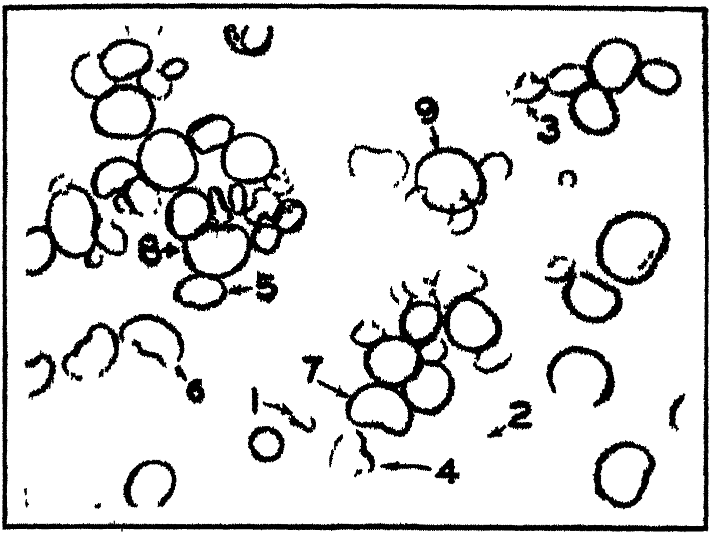


Fig. 12. Various stages in the development of endosperm starch granules—as found in a kernel harvested 12 days after pollination (×600). Numbers (1, 2, 3, etc.) indicate possible order in stage of development.

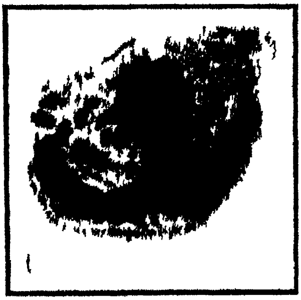


Fig. 13. Endosperm cell containing starch granules which probably are only 1 or 2 days old. Stained with iodine (×600).

cleus" or hilum [6] on which more starch deposits, the form of the developing granule would be dependent on whether the deposition starts at one point with growth around the hilum from that point or whether the deposition starts at two or more points. Figure 13 shows the various shapes of the developing starch granule as they may be observed in the starch cell In so far as one can judge, this cell was probably only a day older than that shown in Figure 10.

In general the growth of the granule is much more rapid in one plane, thus forming (when the "nucleus" is completely enveloped) a disc with a prominent bulge in its middle As the granule grows and approaches maturity, it becomes thicker and more dense with the "nucleus" becoming less prominent.

That the middle portion of the granule is not a "spherical space" or cavity, as is suggested by the "growth by intussusception" theory, may be seen under the microscope by allowing evaporation to take place at one edge of the cover glass, as the immature granules float across the field under the microscope some of them roll over and as they turn edgeways the prominent bulge in the middle may be clearly seen Figure 14 shows some immature granules as they appear when viewed from the edge.

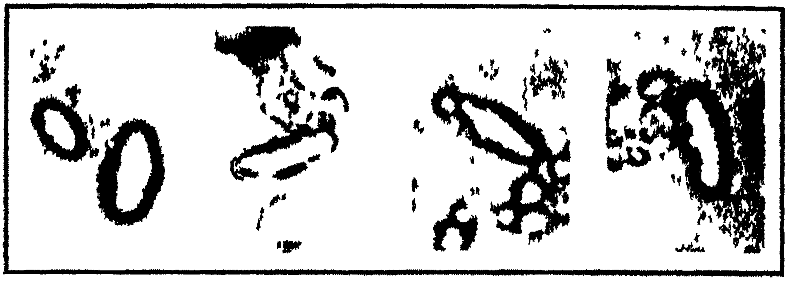


Fig 14 Edgewise views of immature endosperm starch granules showing the bulging nucleus in profile

The bulge in the middle of the granule gives a more distinct birefringence and stains more deeply with iodine than does the surrounding starch (Fig. 11 *D* and *E*) Denniston (1907) suggests that the difference in staining between the inner and outer portions of the starch granule indicate that the outer portion is a transition layer. According to the above observations this seems to be a logical explanation. However, the fact that many waxy starch granules (as found in the waxy sorghums, corn, and barley) have a center which stains blue with iodine (Meyer, 1886, and Lampe, 1931) indicates the possibility of there being considerable difference in the type of starch laid down in a granule at different times and under different conditions.

---

[6] Perhaps this beginning sphere should be called the hilum of the developing granule since the hilum "is said to be the organic center or nucleus around which the granule has grown (Trubell, 1914)

The immature granules are transparent and flexible, stain readily with dilute iodine, and have indistinct birefringence, which suggest a highly hydrated starch; i.e., a semiliquid starch with perhaps relatively few bonds between molecules. With growth the starch granules become less flexible, more dense, and thicker, and their birefringence becomes more distinct. Perhaps these changes in the granule may be explained by a decrease in hydration with a greater number of bonds formed between molecules (the bonds formerly holding water of hydration being available to form bonds between molecules). Deposition of more starch, either by apposition or by intussusception, in such a case would account for the thickening of the granule and also may be a factor in producing more definite birefringence.

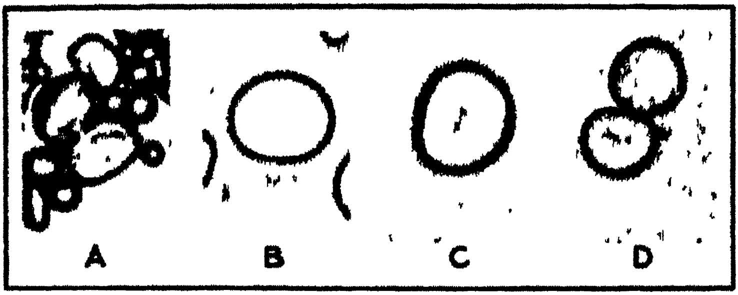


Fig. 15. Endosperm starch granules with bean shaped hila.
A. Immature granule (×660).
B D. Partially gelatinized mature granules (×140).

Some of the immature forms of the developing granule may be observed serving as "nuclei" of older granules. In Figure 15, *A* shows an immature granule with a bean-shaped "nucleus." The "nucleus" of the mature wheat granule is not ordinarily visible under the microscope; however, by careful partial gelatinization it may be differentiated from the surrounding structure. Figure 15 *B*, *C*, *D* shows mature granules gelatinized in water at 55° with the bean-shaped "nuclei" plainly visible.

The Spherical Type of Endosperm Granule. Most mature starches prepared from pure material are composed of granules varying widely in size. Usually, however, all of the granules from a particular material are of one definite type. Wheat, barley, and rye starches are peculiar in that they contain two distinct types of granules: Small *spherical* granules and larger lenticular granules. By manipulating the cover glass, or by allowing evaporation from the edge of the cover glass, so as to cause a rolling movement of the granules of mature wheat starch, the spherical nature of the small granules as contrasted with the well-known lenticular character of the large granules is readily observed.

At about the time that the kernel attained its maximum length (corresponding to stage *G* of Figure 1 which represents the development at about 12 to 14 days after pollination) a few small granules began to appear among the lenticular endosperm granules (Fig. 11 *G*). Up to this time the granule population of a cell was relatively uniform in size. By the time the kernel had reached maturity, these small granules were present in large numbers and could be seen to be spherical. They attained a maximum diameter of about 10 μ as compared to about 35 μ for the lenticular granules (Fig. 11 *I*).

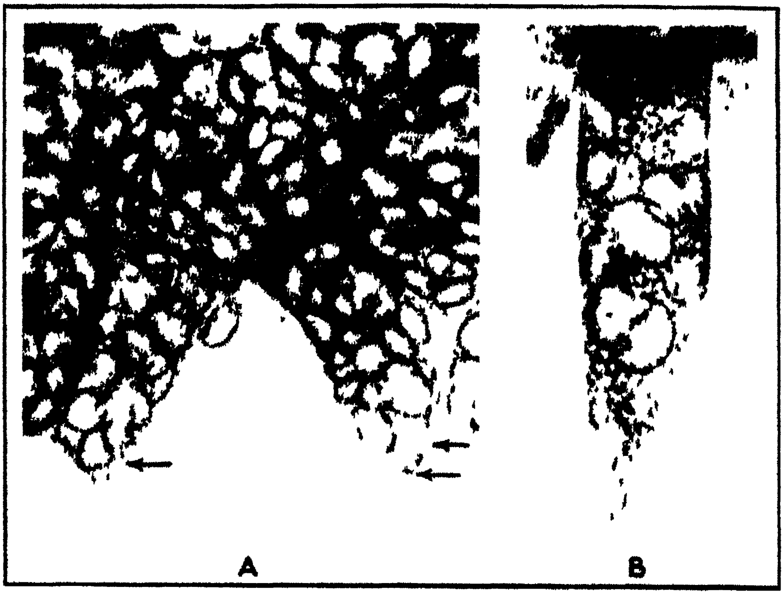


Fig 16 Endosperm cells showing development of spherical granules (×373)
A Showing the beginning deposition of spherical granules—arrows
B Mature cell with available space packed with spherical granules

Figure 16 illustrates the development of the spherical type of granule in the endosperm starch cells. The cells in *A* show the beginning deposition of spherical granules; the lenticular granules had attained complete circular form. Cell *B* was from a mature kernel of wheat showing the small spherical granules completely filling all available space. As may be seen by comparing the size of the starch granules of the cells shown in *A* with those in *B*, the lenticular starch continued to grow during the period of deposition and growth of the spherical granules.

Mature lenticular wheat starch granules quite generally show surface markings similar to those on the granules of Figure 11 *I*. On careful examination under the microscope these appear to be indentations in the surface A comparison of these markings with the appearance of the small granules overlying the large granules in the mature starch cell (Fig. 16 *B*) suggests that the packing of the small granules into all available space in the cell may produce indentations on the large granules which become permanent during the late growth and drying of the kernel.

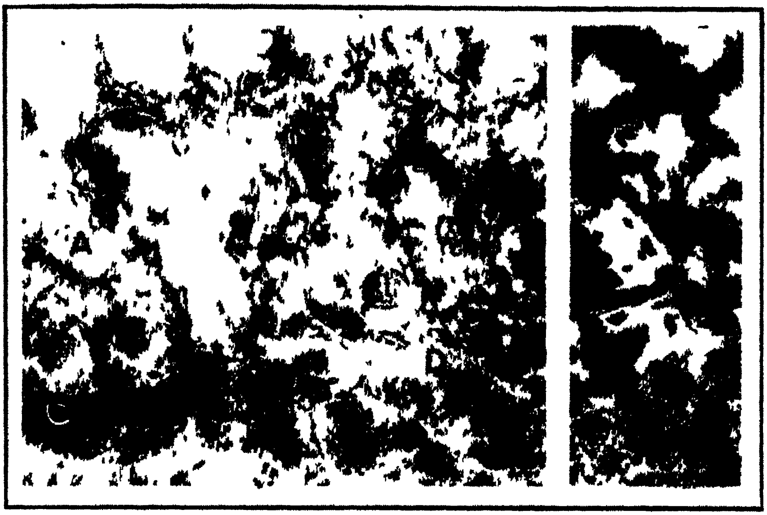


Fig 17 Section of the endosperm 6 days after flowering (×300)
A Cell containing bean shaped granules.
B Aleurone layer
C Newly formed starch cells before starch deposition had started.
Right—similar section stained with iodine

The Function of the Aleurone.[7] During the early development of the wheat kernel, the lenticular type of starch as pressed from an entire endosperm consisted of a mixture of granules in various stages of development. However, on examining cross sections cut from the middle and both ends of a kernel, it was evident that the granules of any particular cell were all quite uniformly developed (until the appearance of the spherical granules), whereas there was a gradation in development of the starch cells from the periphery inward.

---

[7] In the normal course of kernel development the outer or peripheral layer of cells of the endosperm becomes the aleurone of the mature kernel. During the early stages of development this peripheral layer does not have the characteristics generally associated with mature aleurone In this paper the term aleurone is used to designate the peripheral layer of endosperm cells at all stages of development

Figure 17 shows a portion of the cross section of the endosperm of a kernel of wheat harvested six days after pollination The starch granules in the interior of the endosperm had developed to about the stage of the larger granules shown in Figure 11 *C;* the cell *A* contained granules at the "bean-shaped" stage, whereas the cells nearer the periphery contained granules in still earlier stages of development (*D*). Staining a similar section with iodine (Fig. 17—right) shows that the aleurone (*B*) and the next layer within (*C*) contained no starch granules (a few granules may be carried into this area by the microtome in sectioning). The apparent gradation of starch cell development from the periphery to the middle of the endosperm indicates a gradation in age of cells. It is significant that in the cross sections of the immature kernel (Figs. 9, 17, 19, and 20) the individual cells in the first row of cells inward from the aleurone line up with the aleurone cells—a young starch cell corresponding to one or two aleurone cells.

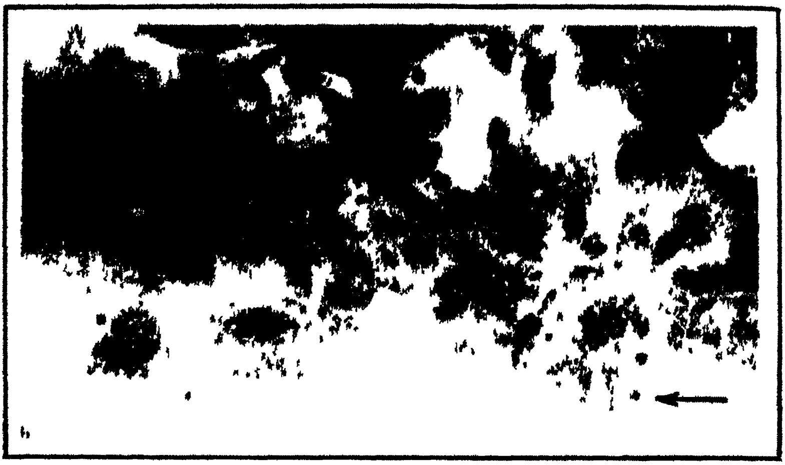


Fig 18 Section of endosperm stained with iodine showing radial cell division in an aleurone cell (×546)
Arrow points to cell in the telophase of division The two daughter nuclei are clearly visible

Gordon (1922) proposed the theory that the aleurone layer during the early growth of the kernel is a meristematic cambium tissue which produces new endosperm cells. Mottier (1921) expressed a somewhat similar view concerning the peripheral region of some other cereals, although he thought that the most active meristematic cells lay just inside the aleurone. The photomicrographs of cross sections of the immature wheat kernel shown in the present paper lend support to the theory that the aleurone during the growth of the grain is a meristematic tissue and that its function is the production of endosperm cells

Apparently the aleurone cells divide both tangentially, producing daughter starch cells, and radially, producing new aleurone cells. Two aleurone cells corresponding to one starch cell would indicate that a radial division took place after the last tangential division (Fig. 19 *B*). Gordon (1922) showed tangential division in her illustrations. Figure 18 shows radial mitotic division taking place in an aleurone cell. This section was stained with iodine and accordingly also shows the range in starch granule development from the aleurone inward.

In the early development of the grain, the only visible evidence of differentiation of the peripheral layer of the endosperm to form the aleurone was the absence of starch (Figs. 9 and 17). As the grain developed, the differentiation of this layer became more evident; it became more dense in appearance due to the deposition of "aleurone granules" (Gordon, 1922). Figure 19 shows a section of a kernel harvested 12 days after pollination. This would correspond to a stage similar to that shown in Figure 1 *E*. By this time the aleurone layer was definitely, visibly differentiated. Most of the first layer of cells just inside the aleurone showed starch granules in the early stages of development, though varying somewhat in age from cell *C* to *D;* this second layer was more developed (or older) than the corresponding layer in the earlier stages of growth (Figs. 9 and 17), indicating that the

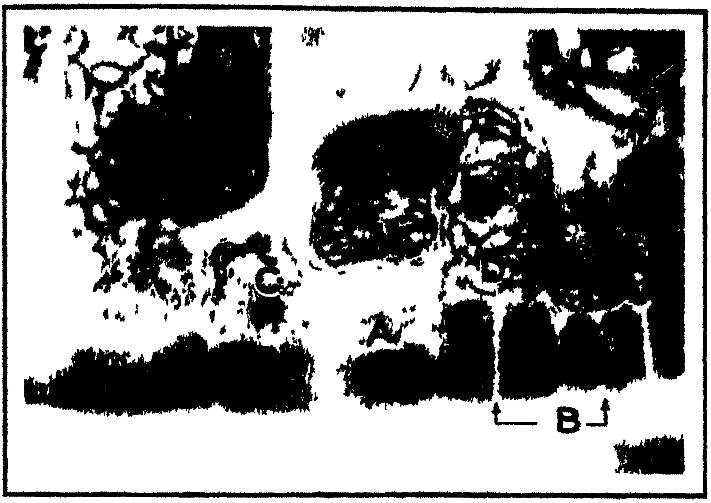


Fig 19 Section of endosperm 12 days after flowering (×330)
(Some cells displaced due to thin sectioning)

A Young starch cell arising from a tangential division
B Pairs of daughter cells produced by radial divisions in the aleurone layer Each pair corresponds to a single starch cell
A, *C*, and *D* illustrate the progressive steps in the differentiation of a starch cell

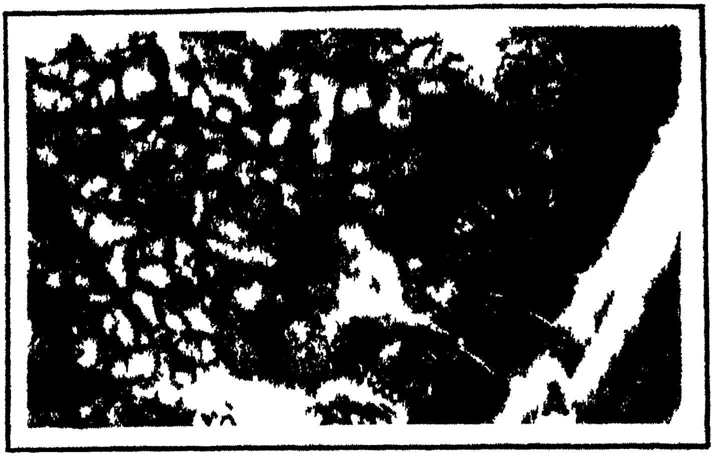


Fig 20 Section of endosperm 14 days after flowering (X330)

A Remains of the nucellus
B Aleurone
C Cell showing beginning deposition of spherical starch granule

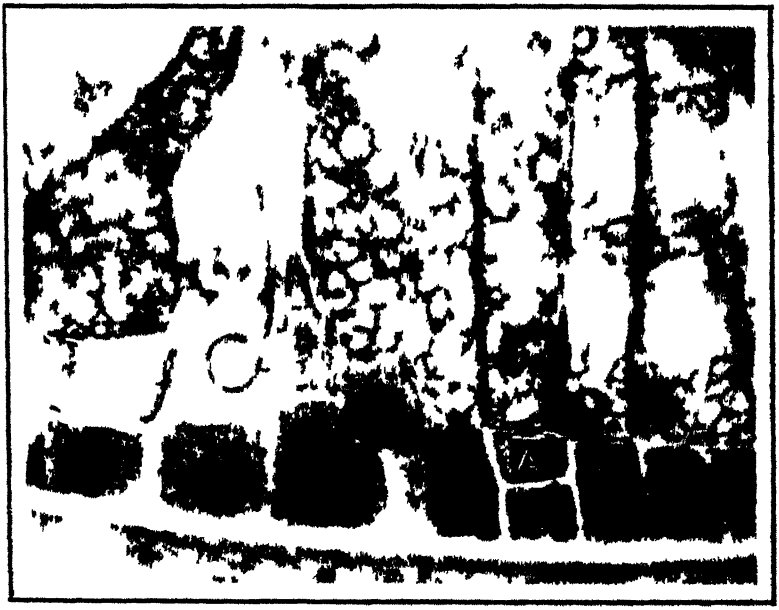


Fig 21 Section of endosperm 16 days after flowering (X287)

A Newly formed starch cell not yet visibly differentiated from the mother aleurone cell
B Newly formed starch cell with starch granules beginning to form

rate of division of the aleurone cells was decreasing with age. Evidence of recent division, however, was still found a few cells had divided so recently (Fig. 19 *A*) that the inner cell still contained no starch but was similar in appearance to its mother cell.

Figure 20 shows a section of kernel harvested about 14 days after pollination. Apparently by this time aleurone cell division had slowed down considerably Cell *C* contains a few newly formed granules of the spherical type of starch.

As may be seen in Figure 21, a section cut from a kernel harvested 16 days after pollination, there is still evidence of recent aleurone cell division at this late stage in the kernel development. Cell *A* was too young to show differentiation from its mother cell, whereas cell *B* had begun to develop starch granules.

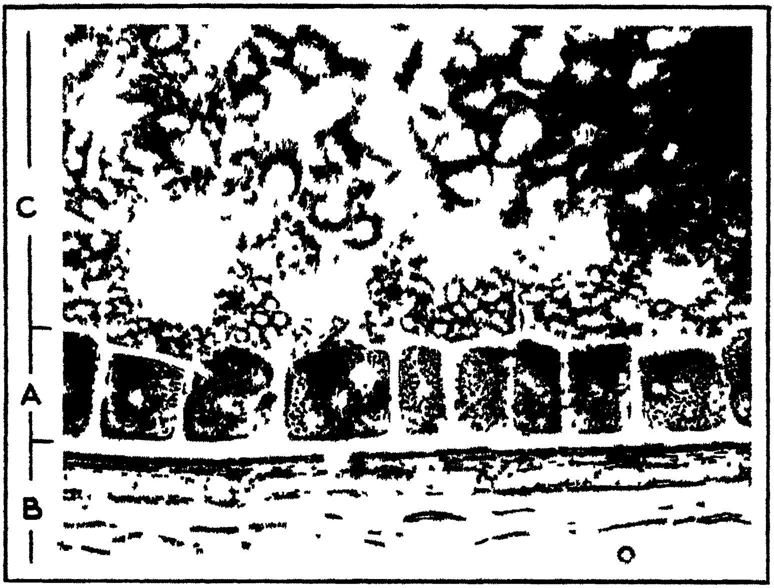


Fig 22 Section of mature wheat kernel showing smaller granules in the layer of starch cells just within the aleurone (X287)

A Aleurone
B Bran
C Starchy endosperm

Even sections cut from mature kernels of wheat show starch cells containing small starch granules just within the aleurone layer (Fig. 22). Apparently the aleurone continues to form new starch cells until so late in the growth of the kernel that the last-formed cells and the granules within the cells do not complete their development before the kernel matures; this seems to apply particularly to those cells at each side of the "furrow" or "crease" of the kernel.

Summary

The development of wheat starch granules in the maturing kernel was followed by means of photomicrographs.

Minute starch granules are present in the unfertilized wheat ovary.

Pericarp starch develops rapidly showing maximum diameter of granules by the 4th or 5th day. However, an enzyme is present in the pericarp which is capable of digesting starch granules; accordingly, the larger starch granules show indications of being attacked by the time they have reached maximum diameter. Pericarp starch practically disappears from the wheat kernel (due to enzymatic digestion) within a couple of days after the kernel attains its maximum length.

Minute endosperm starch granules may be found in the interior of the endosperm at about the time that cell walls form in this tissue, or approximately the 4th or 5th day after the flower has been fertilized. These granules develop into the large lenticular granules which are characteristic of wheat starch. Rye and barley starches also contain this type of granule.

Small spherical granules begin to develop in the cells containing lenticular granules at about the time the kernel attains full length. These granules completely fill the space between the lenticular granules.

Evidence is given supporting Gordon's (1922) contention that the peripheral layer of endosperm cells (the aleurone layer of the mature grain) is a meristematic tissue which produces new endosperm starch cells.

Literature Cited

Badenhuizen, N. P.
1939 Growth and corrosion of the starch grain in connection with our present knowledge of the microscopical and chemical organization. Protoplasma 33: 440–468.

Bice, C. W., MacMasters, M. M., and Hilbert, G. E.
1945 Wheat starch properties in relation to grain maturity. Cereal Chem. 22: 463–476.

Brenchley, W. E.
1909 On the strength and development of the grain of wheat (*Triticum vulgare*). Ann. Botany 23: 117-139.

——, and Hall, A. D.
1909 The development of the grain of wheat. J. Agr. Sci. 3: 195–217.

Buchanan, J. H., and Naudain, G. G.
1923 Influence of starch on strength of wheat flour. Ind. Eng. Chem. 15: 1050–1051.

Denniston, R. H.
1907 The growth and organization of the starch grain. Trans. Wisconsin Acad. Sci. 15: 664–708.

Eckerson, S. H.
1917 Microchemical studies in the progressive development of the wheat plant. Wash. State Coll. Agr. Expt. Sta. Bull. 139.

Evans, J. W.
1941 Microscopic examination of developing corn starch. Cereal Chem. 18: 462–464.

Gordon, M.
1922 The development of endosperm in cereals. Proc. Roy. Soc. Victoria **34**: 105–116.
Grewe, E., and Bailey, C. H.
1927 The concentration of glutenin and other proteins in various types of wheat flour. Cereal Chem. **4**: 230–247.
Harlan, H. V.
1920 Daily development of kernels of Hannchen barley from flowering to maturity at Aberdeen, Idaho. J. Agr. Research **19**: 393–430.
Jensen, G. H.
1918 Studies on the morphology of wheat. Wash. State Coll. Agr. Expt. Sta. Bull. 150.
Jones, C. R.
1940 The production of mechanically damaged starch in milling as a governing factor in the diastatic activity of flour. Cereal Chem. **17**: 133–169.
Lampe, L.
1931 A microchemical and morphological study of the developing endosperm of maize. Botan. Gaz. **91**: 337–377.
MacMasters, M. M., and Hilbert, G. E.
1944 The composition of the "amylodextrin" fraction of wheat flour. Cereal Chem. **21**: 548–555.
McNair, J. B.
1930 The differential analysis of starches. Field Museum Nat. History. Botan. Ser. 9(1). Publication 275.
Meyer, A.
1886 Ueber Stärkekörner, welche sich mit Jod roth färben. Ber. deut. botan. Ges. **4**: 337–362.
Miller, E. C.
1939 A physiological study of the winter wheat plant at different stages of its development. Kansas Agr. Expt. Sta. Tech. Bull. 47.
Mottier, D. M.
1921 On certain plastids, with special reference to the protein bodies of Zea, Ricinus, and Conopholis. Ann. Botany **35**: 349–364.
Percival, J.
1921 The Wheat Plant. Duckworth and Co., London.
Randolph, L. F.
1936 Developmental morphology of the caryopsis in maize. J. Agr. Research **53**: 881–916.
Reichert, E. T.
1913 The differentiation and specificity of starches in relation to genera-species, etc. Part I. Carnegie Institute of Washington. 173.
Stramberg, O. E.
1939 Starch as a factor in dough formation. Cereal Chem. **16**: 769–780.
Teller, G. L.
1938 Plant diastase in evidence as to the formation and structure of starch granules. Plant Physiol. **13**: 227–240.
Thatcher, R. W.
1913 The progressive development of the wheat kernel. J. Am. Soc. Agron. **5**: 203–213.
1915 The progressive development of the wheat kernel. II. J. Am. Soc. Agron. **7**: 273–282.
Trubell, O. R.
1944 Occurrence and varieties of starch. Kerr, R. W. Chemistry and industry of starch. Pp. 1–18. Academic Press, New York.
Weatherwax, P.
1930 The endosperm of zea and coix. Am. J. Botany **17**: 371–380.
Wieler, A.
1938 Ein Beitrag zur Entwicklungsgeschichte der Stärkekörner. Protoplasma **30**: 139–145.
Young, E.
1944 Photomicrographs. Radley, J. A. Starch and its derivatives. Second edition, p. 527. D. Van Nostrand Co., Inc., New York.

THE PROTEOLYTIC ENZYME ACTIVITY OF MILLED FRACTIONS OF WHEAT AND THE EFFECT OF BROMATE IN FLOUR

MARJORIE HOWE and DAVID GLICK[1]

Russell-Miller Milling Company, Research Laboratory, Minneapolis, Minnesota

(Received for publication April 5, 1946)

In a previous communication (1945) the authors considered certain properties of the proteolytic enzymes of milled fractions of wheat, and Howe (1946) showed that when the enzyme activity in germ extract was reduced drastically by hexylresorcinol or sodium fluoride, almost no effect on dough quality could be observed by farinograph studies. This finding was taken as evidence against the proteolytic theory of the effect of redox agents on dough quality.

The present investigation deals with the quantitative distribution of proteolytic activity among the milled fractions, and the effect on the activity of commercial levels of bromate in a straight grade flour. It was the purpose of the latter study to elucidate further the mechanism of bromate action with respect to the proteolytic theory.

Materials and Methods

All milling separations were obtained from the same mix made up entirely of 1945 crop spring wheat. The samples were stored in a refrigerator.

The measurements of proteolytic activity were carried out as follows: The whole wheat, bran, germ, and shorts were ground in a Wiley mill and the material passing a No. 20 sieve was used. A 2-g sample was weighed into a 200-ml Erlenmeyer flask and 10 ml of distilled water was added. The mixture was agitated until a uniform suspension was obtained and it was allowed to stand at room temperature for one hour. Ten ml of $M/7$ sodium acetate was added, followed by 5 ml of 0.75% cysteine hydrochloride and 5 ml of 6% casein, prepared as described previously by the authors (1945). With stirring, 1 N acetic acid was added to bring the pH to 5.0; this usually required 1 ml. Distilled water was finally added to bring the total volume to 35 ml, and four drops of chloroform were added to inhibit the growth of microorganisms. A separate flask with 35 ml of reaction mixture was used for each point on the activity-time curves shown in Figures 1 and 2. With any given reaction mixture one flask was emptied into a centrifuge tube as soon as it was made up, centrifuged for two minutes, at 2000 r.p.m. and 10 ml of the supernatant was titrated for amino groups by the acetone method previously employed (Howe and Glick, 1945). Other flasks were placed in a thermostat at 37°C for various periods up to six hours, after which the liquid was centrifuged and 10 ml of the supernatant was titrated as above. Control experiments were carried out by heating the mixture to boiling under a reflux condenser. The samples were placed in a thermostat for periods up to six hours, and the liquid centrifuged and titrated as previously described. Nitrogen was determined on all of the fractions, and the results calculated to a 14.0% moisture basis.

---

[1] Present address: Division of Physiological Chemistry, Millard Hall, University of Minnesota, Minneapolis 14, Minnesota.

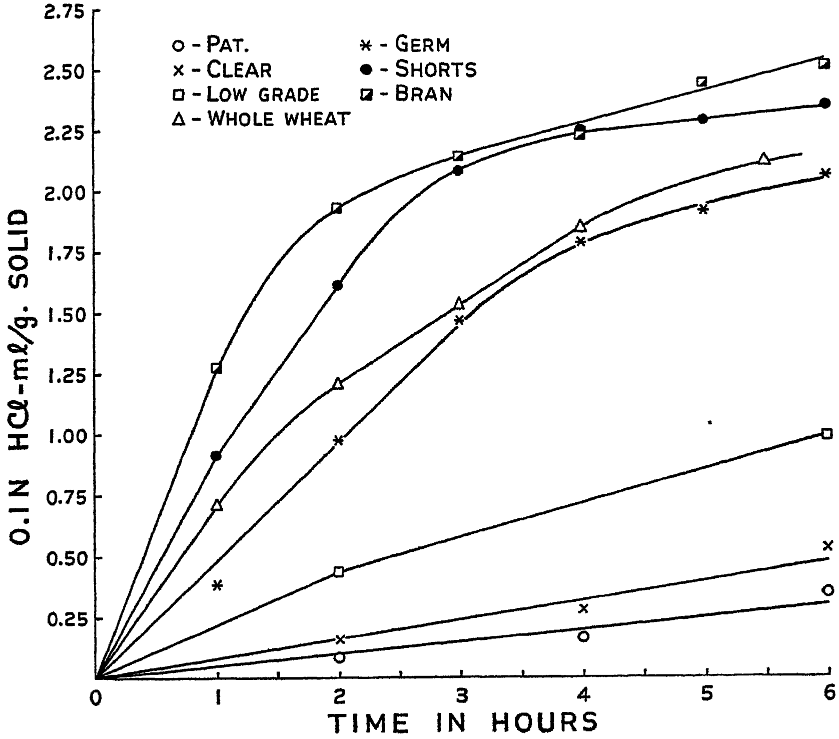


Fig. 1. Rate of proteolysis by milled fractions of wheat per gram of sample.

The bromate experiments were set up in the same manner with and without the casein, but with no cysteine. In all cases the volume was made up to 35 ml with water. These experiments were all performed on a straight grade flour, and amounts of a 4 mg % solution of potassium bromate were added in place of some of the water to give flour-bromate ratios in a range (0.001–0.010% bromate in the flour) covering that used in commercial practice.

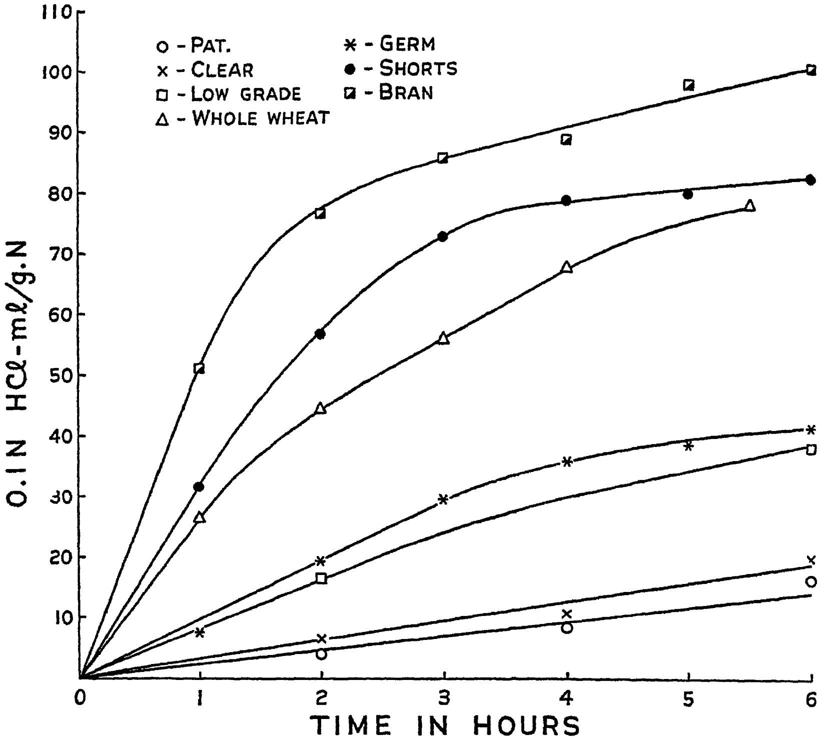


Fig. 2. Rate of proteolysis by milled fractions of wheat per gram of nitrogen.

Results and Discussion

From Figures 1 and 2 it is apparent that the order of the proteolytic activities of the milled fractions, based on either the amount of nitrogen in, or total weight of, sample is: patent < clear < low grade < germ < whole wheat < shorts < bran. The magnitude of the differences between the fractions is also approximately the same in both cases. The greatest deviation is found in the germ whose activity is closer to that of whole wheat when referred to unit weight of total sample; while it is closer to that of low grade when referred to unit weight of nitrogen in the sample. Balls and Hale (1936) reported that proteolysis followed the order: whole wheat < bran < germ. They did not indicate whether the fractions were obtained from the same wheat mix; and unless they were, the results could not be properly compared.

The effect of commercial levels of bromate on the protease activity of straight flour is shown in Table I. Practically no effect was exerted by the bromate on the six-hour titration, and the presence of casein did not measurably increase the value. After 24 hours the effect of the added casein was clearly apparent, but the bromate exhibited no demonstrable inhibition of the activity at the concentrations employed; in fact, a tendency toward activation may be noted at the lower bromate concentrations. Of course, an oxidizing agent such as bromate is known to inhibit the protease action at higher concentrations, but apparently the levels used in commercial practice are well below the range at which inhibition occurs. This finding is in agreement with the conclusions of Read and Haas (1937), and it serves to weaken even more the highly tenuous foundation of the proteolytic theory of the action of bromate on dough properties. As pointed out by many investigators in the past, the proteolysis of wheat and its products is extremely weak. Our experiments again demonstrate that only a negligible percentage of protein can be hydrolyzed in periods far in excess of those required to show an effect on dough property, even under conditions for the maximum enzyme action.

TABLE I

EFFECT OF POTASSIUM BROMATE ON THE PROTEOLYTIC ACTIVITY OF STRAIGHT GRADE FLOUR

	Reaction time	Proteolytic activity [1]			
		Percent potassium bromate in flour			
		0.000	0.001	0.005	0.010
	hours	*ml*	*ml*	*ml*	*ml*
No casein	6	0.10	0.15	0.12	0.12
With casein	6	0.10	0.14	0.15	0.10
No casein	24	0.15	0.25	0.20	0.15
With casein	24	0.34	0.38	0.39	0.28

[1] Expressed as increase in 0.1 *N* HCl required to titrate the amino groups.

It is interesting to consider in this connection the defense offered by Jørgensen (1945), pages 325–336, for the proteolytic theory in the light of the facts revealed by Read and Haas (1937). Jørgensen readily admits the soundness of the experimental evidence presented by Read and Haas to the effect that no depression in nitrogen solubility is apparent in extraction experiments when bromate is added to flour at commercial levels, and only at higher levels can the depression be demonstrated. However, it is Jørgensen's claim that this finding is not in opposition to the proteolytic theory because, according to him, more bromate would be required since the ratio of water to flour was greater in the experiments of Read and Haas than is found in dough, and the presence of yeast in dough adds protease activators which were not present in the reaction mixtures of Read and Haas. It is surprising that Jørgensen should choose to circumvent the serious obstacle placed in the way of the proteolytic theory by advancing arguments which support so clearly the point of view he wishes to refute. For, certainly, dispersing flour in water cannot hinder the interaction of bromate with the reducing substances in the flour; rather it would aid in the process. The critical ratio is that of bromate to the flour which contains the reacting components. And as for the point that reducing materials from yeast were not present in the experiments of Read and Haas, one can be sure that the addition of such reducing substances would only lead to a greater bromate requirement in dough and not a lesser one, as claimed by Jørgensen.

Summary

The relative proteolytic enzyme activities of milled fractions of wheat were determined quantitatively and found to fall in the order: patent < clear < low grade < germ < whole wheat < shorts < bran.

The proteolytic activity of a straight flour was not inhibited by the presence of potassium bromate in concentrations covering the range of those employed commercially. This is taken as additional evidence against the proteolytic theory of bromate action in dough. It was found that flour protease, fully activated by additional cysteine, can hydrolyze only a negligible percentage of the protein in six hours.

Literature Cited

Balls, A. K., and Hale, W. S.
1936 Proteolytic enzymes of flour. Cereal Chem. 13: 54–60.
Howe, M.
1946 Further studies on the mechanism of the action of oxidation and reduction on flour. Cereal Chem. 23: 84–88.
——, and Glick, D.
1945 Activity-pH relationships and inhibition of proteolytic enzyme activity of milled fractions of wheat. Cereal Chem. 22: 502–513.
Jørgensen, H.
1945 Studies on the Nature of the Bromate Effect. Einar Munksgaard, Copenhagen, and Humprey Milford, Oxford University Press, London, 435 pp.
Read, J. W., and Haas, L. W.
1937 Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. Cereal Chem. 14: 752–766.

OPEN TROUGH AND CABINET FERMENTATION OF BREAD SPONGES

BETTY SULLIVAN and W. A. RICHARDS
Research Laboratories, Russell-Miller Milling Company,
Minneapolis, Minnesota

(Received for publication November 14, 1945)

In the usual bakery practice, sponges are fermented in open troughs, preferably in a fermentation room controlled for temperature and humidity. If fermentation is conducted in cabinets, the air space over the sponge is minimized and the process is called cabinet fermentation. Cabinets may be built of a variety of materials from plywood to steel and should be 10 to 12 inches above the maximum height of the sponge. Cabinets are usually provided with doors to allow the entrance of the troughs.

Several bakeries have installed cabinets and have found superior results. Other shops have tried cabinet fermentation and have seen no advantage in the method. Hence, the subject has elicited much interest and heated discussion among bakers.

Johnson (1945) described experiments on small doughs made in the laboratory using different gas mixtures above the fermenting sponge. He found that the rate of expansion and height of the sponge were not affected by changing the concentration of carbon dioxide, oxygen, or nitrogen. However, an atmosphere varying between 15.5 to 25.0% carbon dioxide, 14.0 to 15.5% oxygen, and 61.0 to 69.0% nitrogen for a three-hour sponge produced better bread than the control sponge fermented in air of normal composition. Poor handling doughs resulted from the sponges fermented in high concentrations of oxygen and nitrogen.

Schoonover, Freilich, and Redfern (1946) studied cabinet fermentation on commercial size doughs and made careful measurements of temperature throughout the sponge. The humidity used in the dough room was 96%, which is unusually high. They reported that cabinet fermentation is of no value under controlled conditions in which the room temperature is equal to or slightly higher than the temperature at which the sponge comes from the mixer. However, when the dough room temperature is much cooler than the starting temperature of the sponge, cabinet fermentation showed definite advantages over open trough fermentation. They concluded that the cabinet method has no advantages in shops having accurate temperature and humidity control.

The authors have been interested in the subject of cabinet fermentation since its inception. Numerous comparisons of cabinet and open sponges have been made, but the following experiments should serve to illustrate our results.

Materials and Methods

A spring wheat patent flour of 0.39% ash, 12.50% protein, and 500 mm gassing power (sixth hour) was employed in this work. The same brand of yeast at the same level was used throughout these tests.

A commercial-type sponge and dough procedure was followed using the formula:

	Sponge		Dough		Total-Flour Basis
	lbs	*ozs*	*lbs*	*ozs*	%
Flour	39		26		100.0
Water	23		21		67.7
Yeast	1	5	—		2.0
Yeast food		2	—		0.2
Shortening	1	15	–		3.0
Salt	——		1	5	2.0
Sugar	——		3	4	5.0
Nonfat milk solids	——		2	7	3.75

All standard bakery equipment was used; a water-jacketed, high-speed mixer (65 rpm), troughs 45 inches long, 26 inches wide, and 21 inches deep, divider, rounder, overhead proofer, and molder. The cabinet was built of plywood and fitted securely over the trough. There was a small glass window on top of the cabinet so that the sponges could be readily observed. Sponges at maximum height were 10 to 12 inches below the roof of the cabinet. Sponges were mixed for 5 minutes and taken from the mixer at 78°F (25.6°C). The room temperature was kept uniformly at 80°F (26.7°C), and the humidity maintained quite constant.

Samples for gas analysis were taken approximately one inch above the sponge. A piece of 2 mm glass tubing, fitted at one end with a 2-inch length of rubber tubing, was inserted through a hole drilled in the top of the cabinet. Suitable connections to the gas sampling tube were made outside the cabinet. Gas components were measured in a Fisher Unitized Gas Analyzer.

It has been common experience that the best bread is produced by taking the sponge 30 to 45 minutes after the breaking time and this procedure was generally followed. The temperature of the sponges at maturity varied between 84° and 87°F (28.9° and 30.6°C). Doughs were mixed for 2 minutes after the cleanup and taken from the mixer at 80°F (26.7°C). Dough time was 10 to 15 minutes. Exceptions to the general procedure will be noted. For proofing, the temperature was 94°F (34.4°C), the relative humidity 90%, and the time 1 hour, 10 minutes. Practically no difference was observed in the rate of proof between cabinet and open doughs. One-pound loaves were baked at

430°F (221.1°C) for 35 minutes in a large rotary oven. Volume was measured and reported as the average of eight loaves. Loaves were carefully scored in accordance with the method of the American Institute of Baking (1939). For brevity, only the total score together with pertinent remarks on the machining characteristics and the bread have been recorded.

Comparison of Cabinet and Open Sponges

It was first necessary to ascertain if the cabinet procedure possessed any advantages as compared to the conventional open sponge method and, if so, to determine the reasons for this superiority.

Table I gives pertinent data on the comparisons of the cabinet and open sponges under varying atmospheric conditions. Even when both temperature and humidity of the dough room are regulated, the cabinet doughs always maintain their superiority. All through these experiments the cabinet doughs took an average of 2% more absorption. The sponges had finer and more uniform pores. At remixing they were drier and broke up more easily, allowing a few minutes shorter mixing time. The outstanding difference between cabinet and open sponges is in the machining. Cabinet doughs are drier, more pliable and extensible, and machine more smoothly with less dusting flour. The bread from the cabinet doughs always scored slightly higher than the bread from the open doughs, mainly due to more uniform, finer grain and thinner cell walls. Also, there is a slightly more tender crumb and better crumb color. No significant difference was noted in loaf volume, symmetry, or break and shred between the two procedures.

Release and Retention of Carbon Dioxide above the Sponge

When temperature and humidity are controlled, in addition to the other variables such as formula, fermentation, and mixing time, there is only one possible difference in the conditions of fermentation between cabinet and open sponges. This difference is merely the atmosphere above the sponge for the 30- to 45-minute period between the breaking time and the time of remixing. As can be seen from the gas analyses given in Table I, little or no carbon dioxide is released until the sponge breaks. In a cabinet the carbon dioxide level reaches approximately 20% within 5 minutes after the break and is maintained or increased from that level during the remaining 25 to 40 minutes, resulting usually in a concentration of 20 to 35% carbon dioxide above the sponge. When a cabinet is not used, the air circulation, the size of the fermentation room, and the number of sponges being fermented all affect the amount of carbon dioxide present above the sponge. In current

TABLE I

COMPARATIVE DATA FOR COMMERCIAL SIZE CABINET AND OPEN SPONGES FERMENTED UNDER VARYING ATMOSPHERIC CONDITIONS[1]

Exp. No.	Type of sponge	Relative humidity %	Temp. at maturity[2] °F	Gas analysis time after mixing	Machining quality	Volume cc	Total bread score	Remarks
1	Cabinet	75	84°	15 min. / 1 hr. / 1 hr. 30 min. / 2 hr. / 2 hr. 30 min. / 3 hr. % CO_2 0.0 / 0.0 / 0.1 / 0.7 / 18.4 / 26.8 % O_2 20.8 / 20.8 / 20.8 / 20.7 / 17.1 / 15.0 % N_2 79.2 / 79.2 / 79.1 / 78.6 / 64.5 / 58.2	Smooth, slightly wet.	2790	95.0	
2	Open	55	84°	15 min. / 1 hr. / 1 hr. 30 min. / 2 hr. / 2 hr. 30 min. / 3 hr. / 3 hr. 30 min. / 4 hr % CO_2 0.0 / 0.0 / 0.0 / 0.2 / 0.4 / 10.0 / 13.0 / 16.2 % O_2 20.7 / 20.7 / 20.6 / 20.8 / 20.3 / 18.7 / 18.2 / 17.4 % N_2 79.3 / 79.3 / 79.4 / 79.0 / 79.3 / 71.3 / 68.8 / 66.4	Smooth, slightly wet and soft.	2810	93.0	Open grain.
3	Cabinet	76	84°	2 hr. 15 min. / 2 hr 40 min / 3 hr 15 min % CO_2 0.2 / 6.6 / 31.8 % O_2 20.6 / 19.0 / 13.6 % N_2 79.2 / 74.4 / 44.6	Excellent.	2855	96.5	
4	Open	90	84°	2 hr. 15 min. / 2 hr. 35 min. / 3 hr. 15 min. % CO_2 0.0 / 0.6 / 10.6 % O_2 20.5 / 20.0 / 18.4 % N_2 79.5 / 79.4 / 71.0	Not as extensible as Exp 3	2835	94.0	Sponge was wetter than Experiment 3. Pores not as fine or smooth.
5	Open	100	86°	2 hr. 45 min. / 3 hr. / 3 hr 15 min. % CO_2 10.0 / 12.6 / 11.0 % O_2 11.6 / — / — % N_2 78.4 / — / —	Closer to cabinet but shorter at rounder and molder. Trifle wet at molder.	2540	94.5	Slightly open grain, wet texture.
6	Cabinet	80	85°	2 hr. 45 min. / 3 hr / 3 hr 15 min % CO_2 27.0 / 27.6 / 27.6	Excellent—drier than Exp. 5.	2600	97.0	More tender crumb than Experiment 5.
7	Cabinet with oxygen.	86	86°	1 hr. / 2 hr. / 2 hr. 25 min. / 2 hr. 40 min. % CO_2 0.0 / 18.0 / 32.5 / 34.2 % O_2 52.5 / 40.8 / 32.3 / 34.4 % N_2 47.5 / 41.2 / 35.2 / 31.4	Wet out of rounder and at end of intermediate proof. Short at molder.	2750	89.0	Sponge was sticky, coarse, and "dead." Absorption at dough stage was 3% below standard. Bread had open, coarse grain, crumbly texture, and poor flavor and taste.

TABLE I—*Continued*

Exp. No.	Type of sponge	Relative humidity %	Temp. at maturity[2]	Gas analysis time after mixing	Machining quality	Volume cc	Total bread score	Remarks
			°F					
8	Cabinet with nitrogen.	80	84°	1 hr. 2 hr. 2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 0.2 0.6 13.5 16.4 19.2 %O_2 20.0 16.8 15.9 14.6 14.0 %N_2 79.8 82.6 70.6 69.0 66.8	Soft and sticky at divider and molder.	2680	91.0	7% lower absorption. Longer mixing time at dough stage until smooth. Slightly open grain.
9	Open in CO_2 filled room.	78	84°	1 hr. 2 hr. 2 hr. 30 min. 3 hr. 15 min. %CO_2 8.6 8.3 9.2 8.4 %O_2 18.8 18.7 18.8 18.8 %N_2 72.6 73.0 71.0 72.8	Smoother than the regular open sponge, still not as dry or mellow as cab. doughs.	2630	95.5	Better grain and more tender crumb than open sponges without CO_2 addition.
10	Cabinet in CO_2 filled room as in Exp. 9.	72	84°	Atmosphere in room as in Exp. 9. Inside cabinet at 3 hr. 15 min. %CO_2 32.4 %O_2 14.0 %N_2 53.6	Excellent.	2740	96.5	Very tender crumb.
11	Open in CO_2 filled room.	72	84°	1 hr. 2 hr. 2 hr. 45 min. 3 hr. 15 min. %CO_2 6.8 11.0 17.4 19.4 %O_2 19.0 18.2 17.0 16.6 %N_2 74.2 70.8 65.6 64.0	Almost equal to cabinet doughs. Better machining than Exp. 9.	2640	95.0	
12	Cabinet in CO_2 filled room as in Exp. 11.	78	86°	Atmosphere in room as in Exp. 11. Inside cabinet at 3 hr. 15 min. %CO_2 43.8 %O_2 11.2 %N_2 45.0	Dry, mellow, elastic, better handling than Exp. 10.	2790	96.5	
13	Open in CO_2 filled room.	89	86°	1 hr. 2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 31.4 21.5 20.6 20.6	Excellent—equal to cabinet doughs.	2560	96.5	Slightly open grain.
14	Open in CO_2 filled room.	62	86°	1 hr. 2 hr. 2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 6.2 7.6 32.0 45.4 27.6[3] %O_2 19.3 18.9 13.8 10.8 14.0 %N_2 74.5 73.5 54.2 43.8 58.4	Excellent—equal to cabinet doughs.	2600	96.5	

---

[1] Room temperature 80° F (26.7° C).
All sponges were mixed 5 minutes at 65 rpm.
All sponges were 78° F (25.6° C) when taken from the mixer, except in Exp. 7 where temperature was 77° F (25.0° C).
All doughs were mixed 2 minutes after cleanup.

[2] 84° F = 28.9° C; 85° F = 29.4° C; 86° F = 30.0° C.

[3] Tank went empty at 3 hours 5 minutes.

practice this amount of carbon dioxide is much less than that held above the cabinet sponges; perhaps 5 to 10% carbon dioxide would be a fair estimate. If the fermentation room has a low ceiling, if there is only slight air circulation, or if there is little space relative to the weight of dough being fermented, there will be relatively more carbon dioxide in the atmosphere than would otherwise be the case. As an illustration, on a laboratory scale, doughs made from 10 pounds of flour (6 pounds of flour in the sponge) were fermented by the cabinet and open procedures (with 2% yeast and the same formula as given for the large doughs) in a Bailey-Walker cabinet, maintained at 80°F (26.7°C) and 75% relative humidity. In the Bailey-Walker cabinets there is quite an extensive air circulation. The louvers were open just sufficiently to allow the temperature and humidity to be held constant. Gas analyses gave the results shown in Table II.

TABLE II

COMPARATIVE COMPOSITION OF ATMOSPHERE OVER LABORATORY SCALE CABINET AND OPEN SPONGES FERMENTED IN A BAILEY-WALKER CABINET (MAINTAINED AT 80°F (26.7°C) AND 75% RELATIVE HUMIDITY)

	Cabinet	Open	Cabinet	Open
Breaking time of sponge	2 hr. 30 min.	3 hr 40 min.	2 hr. 45 min	3 hr. 45 min.
Gas analyses at:				
2 hr. 25 min.	%CO_2 1.0 %O_2 20.6 %N_2 78.4	%CO_2 0.0 %O_2 20.8 %N_2 79.2	—	—
2 hr. 30 min.	—	—	%CO_2 0.6 %O_2 20.0 %N_2 79.4	—
2 hr. 35 min.	%CO_2 12.5 %O_2 17.7 %N_2 69.8	—	—	—
2 hr. 45 min.	—	—	%CO_2 2.3 %O_2 20.0 %N_2 77.7	—
3 hr.	—	—	%CO_2 22.4 %O_2 15.9 %N_2 61.7	—
3 hr. 15 min.	—	—	%CO_2 23.4 %O_2 15.8 %N_2 60.8	—
3 hr. 45 min.	%CO_2 19.5 %O_2 14.9 %N_2 65.6	%CO_2 0.2 %O_2 20.7 %N_2 79.1	—	%CO_2 8.0 %O_2 19.0 %N_2 73.0
4 hr. 30 min.	%CO_2 25.4 %O_2 15.4 %N_2 59.2	%CO_2 1.5 %O_2 20.3 %N_2 78.2	—	%CO_2 1.0 %O_2 20.4 %N_2 78.6

As usual, gas samples were taken approximately 1 inch above the sponge. The highest carbon dioxide obtained above any of the open sponges was 8%. A few minutes later most of the carbon dioxide was swept out. On the other hand, the cabinet doughs naturally confined the liberated carbon dioxide above the sponge. In a series of four such comparisons, the open sponge did not break until about 3 hours, 40 minutes, even though the humidity was 75%. There was sufficient circulation of air to cause some thickening of the surface layer of the sponge, although there was no noticeable crusting. The best bread was produced by allowing a sponge time of 4½ hours for the open sponges. The cabinet sponges broke between 2 hours, 30 minutes to 2 hours, 45 minutes and the best bread was produced with a sponge time of 3 hours, 15 minutes. The cabinet dough had superior handling properties whether the sponges were fermented for 3 hours or 4 hours. Conditions such as the size of the doughs and an air circulation sufficient to cause crusting are obviously different from those maintained in good commercial practice. These data are cited merely to show the effect of too much air circulation on the carbon dioxide content of the air above the sponges. In the large commercial doughs, on the other hand, breaking time is the same for both cabinet and open fermentations if the sponges are fermented at a high enough humidity.

In the fermentation rooms of well-equipped bakeries, there is not usually the extensive forced air circulation that exists in our standard laboratory fermentation units. On laboratory size doughs, results more in accord with cabinet fermentation are, of course, achieved when sponges are covered.

Effect of Relative Humidity on Breaking Time of the Sponge

When open sponges are fermented in a room where the humidity is under 70%, the sponge takes a longer time to break and the breaking time is in direct relation to the relative humidity. A low relative humidity in the dough room necessitates a longer sponge time if judged by the time of break. From 70 to 100% relative humidity the breaking time is about the same. Apparently greater humidity makes the surface of the sponge weaker and more pliable; hence it breaks more quickly and evenly with a given amount of pressure to permit the release of the gas generated during fermentation. Experiments 1 through 5 in Table I illustrate this. The open sponge in Experiment 2 at 55% relative humidity broke unevenly in 3 hours, 5 minutes; whereas the cabinet sponges (75 to 82% humidity) and the open sponges, fermented at 75 to 100% humidity, broke in practically the same time, 2 hours, 30 minutes, to 2 hours, 40 minutes. It is recognized that the optimum sponge time need not be estimated by such an arbitrary procedure as the one we have followed, for, provided a certain minimum time is given, bread quality is not greatly influenced by variations in sponge time.

One great advantage of cabinets lies in their ability to control humidity in small shops having no well-regulated fermentation room. A dough put in a cabinet directly from the mixer gives off enough moisture to keep the humidity at a fairly constant and optimum level. Also, as Schoonover, Freilich, and Redfern (1946) have shown, cabinets effect some degree of temperature control in rooms where the temperature is below or above the temperature of the sponge. Since many small and medium size shops have inadequate control of temperature and humidity, the use of cabinets to effect some means of control of these important factors is of great value.

Effect of Carbon Dioxide, Oxygen, and Nitrogen Atmospheres over the Sponge

Comparisons between cabinet and open sponges indicated that there could be no factor responsible for the differences observed in dough properties except the concentration of gases above the sponges between the breaking time and the time of remixing—a period of 30 to 45 minutes. At first thought it seemed hardly likely that a gas could diffuse to any extent into the sponge, due to the positive pressure of the carbon dioxide being liberated as a result of fermentation. Rather, it was believed that if the carbon dioxide tension on the outside of the sponge was increased, it might prevent some diffusion of the carbon dioxide from the dough, thus building up, after the initial liberation, a greater concentration of the gas inside the dough. Then it would be possible that the higher amount of carbon dioxide might exert an influence on certain enzyme reactions occurring during the later stage of the sponge fermentation.

In order to secure more information on the permeability of the dough to gases, fermentation was conducted in atmospheres higher than normal in both oxygen and nitrogen. Cabinets were placed over the sponges and the gases introduced into the cabinet immediately after the sponges were taken from the mixer. Results of the gas analyses and the baking data are recorded in Table I, Experiments 7 and 8.

When oxygen was used in the cabinet, the sponge was coarse, sticky, and "dead." At the dough stage more flour was required for the same amount of water, resulting in 4% lower total absorption. The dough was wet out of the rounder and at the end of the intermediate proof. The dough coming from the molder was short. The bread exhibited an excellent oven spring, but had an open grain and a crumbly texture. The aroma, flavor, and taste were flat. Gas analyses at the end of the sponge period demonstrated as high a carbon dioxide content as was found with the regular cabinet sponge. However, the oxygen content of the gas above the sponge was about double and the nitrogen approximately half that shown by the cabinet sponge.

When nitrogen was used in the cabinet, absorption at the dough stage was even less than with the cabinet sponge fermented in the atmosphere containing excess oxygen and 7% lower than the regular cabinet sponge. It was found necessary to mix 6 minutes longer than normally to secure a smooth dough. The dough was soft and sticky through the machines. Although oven spring was good, the loaf representing the sponge fermented in excess nitrogen gave an open grain, a gummy texture, and very poor aroma, taste, and flavor. The atmosphere above the sponge during the last 30 minutes of the sponge period showed a slightly lower carbon dioxide and oxygen content; the nitrogen percentage was higher throughout the sponge period. Some objection might be raised because oxygen and nitrogen were introduced at the beginning of the sponge period. However, similar experiments in which carbon dioxide was introduced at the start of the sponge period gave results equal to, or better than, the usual cabinet doughs.

These experiments demonstrate that the sponge must be permeable to the gases above it and that high concentrations of nitrogen and oxygen cause damage to the physical characteristics of the dough and the resultant bread, as Johnson (1945) has previously shown on smaller doughs.

It is interesting to note, in this connection, the results reported by Baker and Mize (1937) on the effect of mixing doughs in vacuum and in the presence of various gases. They found that doughs mixed in atmospheres of hydrogen and nitrogen became sticky and soft on long mixing; doughs mixed under oxygen developed, in addition, pronounced shortness. However, normal bread was produced from the doughs subjected to mixing in vacuum or in the presence of hydrogen or nitrogen. Doughs mixed in the presence of oxygen gave improvement followed by pronounced deterioration.

Experiments were then conducted to see if open sponges fermented in a room filled with higher than a normal concentration of carbon dioxide gave results similar to the cabinet procedure (Table I, Experiments 9 to 14 inclusive). Carbon dioxide was introduced by attaching a tank to the humidifying system of the fermentation room which was 7 feet high; 5 feet, 9 inches long; and 6 feet, 2 inches wide. Gas samples were taken at the level of the trough by means of tubing inserted through the roof of the room. In Experiment 9 the carbon dioxide in the room varied from 8.3 to 9.2% throughout the sponge period.

A cabinet sponge placed in the room at the same time showed 32.4% carbon dioxide when the sponge was taken. This open sponge was slightly smoother than the regular open sponges but was not as dry and mellow in handling as the regular cabinet dough. The cabinet dough fermented in the room at the same time had excellent machining quality. Experiments 11 and 12 were conducted at a higher level of carbon dioxide. During the last 30 minutes of the sponge period, between 17.4 and 19.4% carbon dioxide was maintained. The open sponge was almost equal to the cabinet sponges in machining and bread quality. The cabinet sponge fermented at the same time in this carbon dioxide-filled room attained a level of 43.8% carbon dioxide. This was the highest figure found in a cabinet sponge and the dough had the best machining characteristics of all the cabinet doughs. In Experiment 13 the carbon dioxide concentration was again increased. At the end of 1 hour it was 31% and thereafter, during the critical period, about 21%. The dough was equal to the cabinet sponges. In Experiment 14 the carbon dioxide was maintained at a still higher level—between 28 and 45% from 2 hours, 45 minutes to 3 hours, 15 minutes. This dough was also equal to that obtained from the cabinet procedure in handling characteristics and in bread quality; thus it would appear that approximately 20% carbon dioxide, with proportionate lowering of oxygen and nitrogen, during the last 30 to 45 minutes of the sponge time is the critical level for the production of excellent machining and superior bread.

Under the conditions of these experiments it was not feasible to ferment a dough in a room where the carbon dioxide was maintained at a high enough concentration for only the last 30 to 45 minutes of the sponge period. In order to build up a sufficient concentration of carbon dioxide in the dough room, it was found necessary to introduce the carbon dioxide from the beginning of the sponge time. However, a rather interesting experiment was tried in which two sponges were fermented under exactly the same conditions of mixing, fermentation time, and temperature, one open and the other with a cabinet on top of the trough. At 2 hours, 25 minutes (just before the break) the cabinet was taken off the cabinet sponge and put on the open sponge. A sponge time of 3 hours, 15 minutes was given both doughs. It was found that the sponge that was covered with the cabinet during the last 50 minutes had superior machining characteristics to the sponge that was open during the last 50 minutes but covered prior to that time.

The machining characteristics comprise the outstanding difference between doughs made from cabinet and open sponges, and it is unfortunate that they cannot be properly evaluated by any single chemical or physical means, but must be judged by the feeling of the dough at the divider, rounder, and molder. Since the farinograph and the extensograph are the only means available to record certain differences in the physical properties of doughs, the following experiments were conducted.

Farinograph and Extensograph Measurements

What change does the higher concentration of carbon dioxide cause during the short interval it is present above the fermenting sponge? As the first step in elucidating this problem, extensograms and farinograms were made on the sponges, and on the doughs after remixing. A different flour from that previously employed was used in this series of experiments, but it showed the same analyses as the flour previously reported. The formula was the same as that described for the experiments listed in Table I and both open and cabinet sponges and doughs were handled at the same absorption. The open sponges were fermented in the small dough room maintained at a relative humidity of 82 to 85% and a temperature of 80°F (26.7°C). Sponges were taken from the mixer at 78°F (25.6°C) after a 5-minute mix and the doughs were mixed for 2 minutes after the cleanup. The cabinet doughs needed less mixing. This procedure was justified because in usual commercial practice doughs are mixed for a definite time after the cleanup. The fermentation times chosen for the open sponges were 3¼, 4, and 4¾ hours, and the best bread was produced with the 4-hour sponge time. Two cabinet doughs were compared with the open sponges, using sponge times of 2 hours, 55 minutes and 3 hours, 15 minutes. Previous experiments with other flours had shown that 3 hours, 15 minutes was ample time for a cabinet sponge. The relative humidity inside the cabinet was 85%. The baking data are presented in Table III.

Duplicate farinograph and extensograph curves were conducted on all samples and good checks were obtained. A weight of 480 g was used for the farinograph curves and 150 g for the extensograms. Farinograms were made on pieces of the sponge taken just before remixing and again on the dough immediately after remixing. The samples for the extensograms were rounded and molded in the usual fashion. Measurements were made immediately on the rounded and molded sponge, but on the remixed dough a rest period of 38 minutes was given. This interval was chosen because it corresponds closely to the time actually taken between the remixing and molding of the large doughs. It is evident that the rounding and molding manipulations in the extensograph change the state of the dough and measure certain physical characteristics of the dough relatively at rest as compared with the farinograph where the gluten strands are subjected to continuous mixing. The length of the extensogram indicates the extensibility, and the height of the curve indicates the resistance of the dough to extension, or the energy necessary to stretch the dough. For best breadmaking results, a proper balance should exist between the length and height of the curve. The interpretation of farinograms is too well known to discuss here.

Figure 1 illustrates the results obtained with the farinograph and the extensograph. The farinograph curves A, B, and C on the open sponges showed the highest consistency and the greatest strength on the 3¼-hour sponge. The 4- and 4¾-hour sponges became progressively weaker, as shown by both the amplitude and the breaking point of the curves. The curves on the 2 hour, 55 minute and 3¼-hour-cabinet sponges (D and E) and the 3¼-hour open sponge (A) were not significantly different, D showing somewhat more strength. Farinograph curves I and J on the cabinet doughs, however, show a definitely higher consistency than the curves (F, G, and H) on the doughs made from all the open sponges. This was not due to the few minutes shorter mixing time of the cabinet sponges at the dough stage, since other experiments have shown that a high consistency on the farinograph curves was obtained when the mixing times on both open and cabinet doughs were identical. Numerous comparisons of laboratory or commercial size cabinet and open sponges under controlled conditions of temperature and humidity have always shown that a drier dough is given by the cabinet method.

At the end of the fermentation period, all sponges are "short"; this is illustrated by the extensograph curves K through O. The curves on the open sponges all indicate about the same extensibility. The height of the curves decreased progressively from 360 to 310 to 280 units as the fermentation time increased. Curve O for the cabinet sponge with a fermentation time of 3 hours, 15 minutes exhibited the most extensibility and the greatest resistance to extension (420 units) of all the curves. The cabinet sponge fermented for 2 hours, 55 minutes, however, gave a curve (N) very similar to the 4-hour open sponge, the only difference being that the cabinet curve was 20 units higher. The extensograms on the doughs are much easier to interpret since conditions more closely parallel the state and time of the dough at actual machining. The extensograph curves S and T of both cabinet doughs gave a greater area than the curves P through R obtained from any of the open sponges. Results in machining verify these measurements since the cabinet doughs machined more smoothly and were drier and more mellow than any of the open sponges.

These results, in conjunction with the baking data given in Table

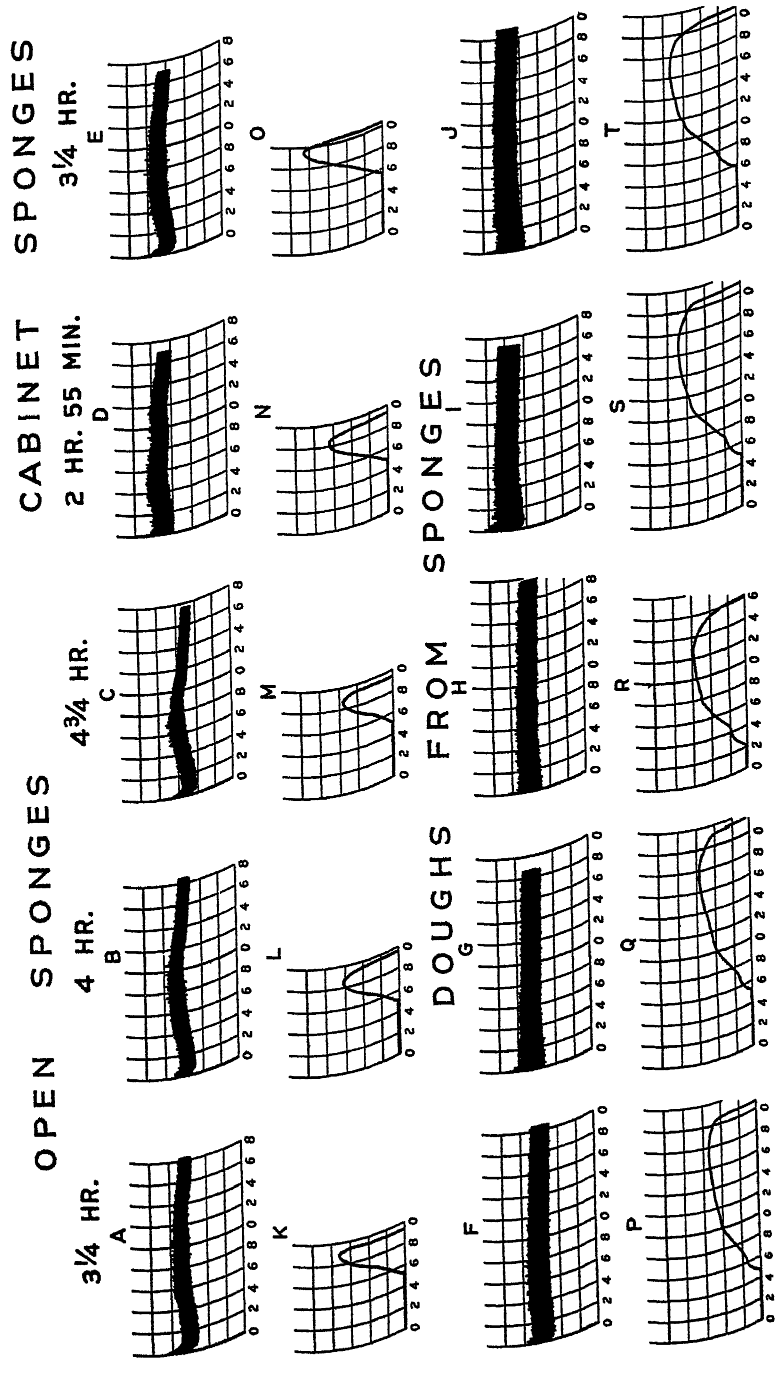


Fig 1 Farinograph and extensograph curves of sponges and doughs

III, substantiate the conclusion that shorter sponge times are possible when using the cabinet procedure.

Farinograph and extensograph curves were also made on the sponges fermented in oxygen and nitrogen and reported in Table I, but space does not permit their inclusion. It was interesting to note that both the farinograms and extensograms on the doughs made from sponges fermented under excess oxygen and nitrogen indicated poor handling properties.

TABLE III

COMPARATIVE BAKING DATA FOR CABINET AND OPEN SPONGES EMPLOYED IN EXTENSOGRAPH AND FARINOGRAPH STUDIES[1]

Exp. No.	Type of sponge	Sponge time	Temp. of sponge at maturity[2] °F	Machining quality	Volume cc	Total bread score	Remarks
15	Open	3 hr. 15 min.	85	Dough mixed smooth but was a trifle soft. No sign of shortness in mixer but was short at divider. Recovered in overhead proofer. Smooth and pliable at molder. Normal proof.	2530	90.5	Crumb was short and slightly gummy.
16	Open	4 hr.	87	Dough mixed smooth but was a trifle soft. No shortness in mixer or at divider. Molded somewhat tough. Not as smooth as usual doughs. Normal proof.	2630	92.5	Slightly open grain.
17	Open	4 hr. 45 min.	87	Sponge was a trifle soft. Dough mixed smooth, soft, and a trifle wet. Some shortness at divider. Molded a little tough. More gas than usual in dough at molder.	2790	90.5	Slightly open grain with a few large holes. Wet and crumbly texture.
18	Cabinet	2 hr. 55 min.	84	Sponge shorter and drier than open sponges. Dough mixed smooth in 4 min. less than open sponges. Machined easily. Dry at divider. Molded very smooth.	2595	95.5	Good grain, thin cell walls, silky texture.
19	Cabinet	3 hr. 15 min.	85	Sponge shorter and drier than open sponges. Mixed smooth in 5 min. less mixing than open sponges. Machined easily. Dry at divider and molder.	2630	95.5	Slightly open grain, thin cell walls, silky texture.

[1] Room temperature, 80°F (26.7°C); relative humidity of dough room, 82 to 85%; relative humidity inside cabinet, 85%. Breaking time of all sponges was between 2 hours, 30 minutes and 2 hours, 40 minutes.

[2] 84°F = 28.9°C; 85°F = 29.4°C; 87°F = 30.6°C.

Hydrogen Ion Concentration of Open and Cabinet Sponges

Although it would not be expected that there would be a difference in the hydrogen ion concentration of the open and cabinet sponges, since there appeared to be no difference between them until the breaking point, this factor was checked on the two sponges fermented under identical conditions of formula, temperature, and time. The only difference, a negligible one, was that the relative humidity inside the cabinet was 83%, while in the dough room, where the open sponge was fermented, it was 76%. Glass and calomel electrodes were inserted in the sponge and the changes in hydrogen ion concentration with time were measured with a Cambridge potentiometer. Results were as follows:

Time after mixing sponge	pH Open	 Cabinet
Initial	5.7	5.7
15 min.	—	5.5
25 min.	5.6	—
45 min.	5.3	5.3
1 hr. 15 min.	5.2	5.3
1 hr. 50 min.	5.1	5.2
2 hr. 5 min.	—	5.1
2 hr. 20 min.	5.1	5.1
3 hr.	5.1	5.1
3 hr. 15 min.	5 1	5.1

It is evident that no difference in hydrogen ion concentration or its rate of change is responsible for the superior machining of the cabinet doughs.

Carbon Dioxide Production under Different Gas Atmospheres

So far there is ample proof that the only condition which could cause the differences observed in the machining of the dough and in the quality of the bread between cabinet and open sponges is the higher concentration of carbon dioxide present above the cabinet sponge during the interval between the breaking of the sponge and its remixing. What is the mechanism of the action of this increased level of carbon dioxide on the sponge? There is a possibility that during this last stage of the sponge fermentation the higher level of carbon dioxide confined over the cabinet dough causes a more rapid generation of gas. This higher concentration of carbon dioxide may then act favorably on certain enzyme systems or on the gluten itself.

To obtain more accurate information on the effect of various gases on the rate of carbon dioxide production by the action of yeast on flour, this phase of the subject was investigated using the Warburg apparatus. Details concerning the principle and the operational techniques have been described by Dixon (1943) and Umbreit, Burris, and Stauffer (1945). Since a free exchange of gases is essential in employing any type of respirometer, it is necessary to employ more dilute suspensions than exist in dough. The suspensions were made up as follows: 50 ml of water or buffer were added to 10 g of the short patent flour employed for the baking procedures, and the suspension was thoroughly mixed for about 2 minutes with a mechanical stirrer. A quantity of 2 ml was used. A volume of 1 ml of the yeast suspension (0.4 g fresh compressed yeast made up to 50 ml with water or buffer) was employed, thus giving a concentration of about 2% yeast based on the flour weight. In all experiments blanks were conducted on the flour and yeast suspensions separately. Each of the blanks was made up to 3 ml with water or buffer and the sum of the two blanks was subtracted from the readings of the flour-yeast mixture. The yeast solution was tipped into the flour suspension from the side arm after 15 minutes' equilibration. A temperature of 37°C was used throughout. In all the experiments where the fermentation was studied with gases other than air, the three flasks with flour alone, yeast alone, and flour and yeast were gassed for 10 minutes before placing them in the bath. The gas was bubbled through water before its introduction in the flasks. A similar series with air was always conducted at the same time. The manometers were then set at 150 after equilibration and readings of the carbon dioxide liberated taken at suitable intervals. Figure 2 illustrates average results obtained using water, rather than a buffer, with flour-yeast mixtures fermented in air, carbon dioxide, nitrogen, and oxygen. Water was used because it simulates more closely the conditions present in dough.

It would be expected that the hydrogen ion concentration of the flour-yeast suspensions gassed with carbon dioxide would be lower than the samples gassed with oxygen or nitrogen. The flour suspension in the main compartment and the yeast suspension in the side arm were gassed for 10 minutes in the usual fashion with carbon dioxide, oxygen, and nitrogen. An air control was used for comparison. The contents of the side arm were added to the flour suspension and the hydrogen ion concentration measured immediately with microelectrodes. Results were as follows:

	pH
Flour extract alone	5.82
Flour-yeast with air	5.79
Flour-yeast with nitrogen	5.66
Flour-yeast with oxygen	5.64
Flour-yeast with carbon dioxide	5.06

The higher hydrogen ion concentration of the flour-yeast mixtures fermented in air or oxygen as compared to carbon dioxide explains the delayed start of the curves in air and in oxygen. Until the water is saturated with the carbon dioxide produced by fermentation, there is no liberation of gas. The solubility of carbon dioxide at 37°C is 0.57 ml carbon dioxide per ml water, which is significantly higher than the other gases employed. Flour-yeast suspensions in water fermented under carbon dioxide and nitrogen gave increased amounts of carbon dioxide as compared to air and oxygen, fermentation in nitrogen being much faster owing, perhaps, to the more rapid diffusion of this gas.

Because of the differences in the initial hydrogen ion concentration of the flour-yeast-water mixtures, it was of interest to repeat the experiments substituting a sodium acetate-acetic acid buffer of hydrogen ion concentration 5.0 for the water. Both the flour and yeast suspensions were made up with this buffer, which had approximately the same hydrogen ion concentration as existed at the start of the carbon dioxide experiment and was also the hydrogen ion concentration found at the end of all the Warburg experiments. Figure 3 illustrates the average results. Because of the lessened solubility of carbon dioxide at the lower hydrogen ion concentration, there is not the usual induction period found when water is used as the medium in the air and oxygen

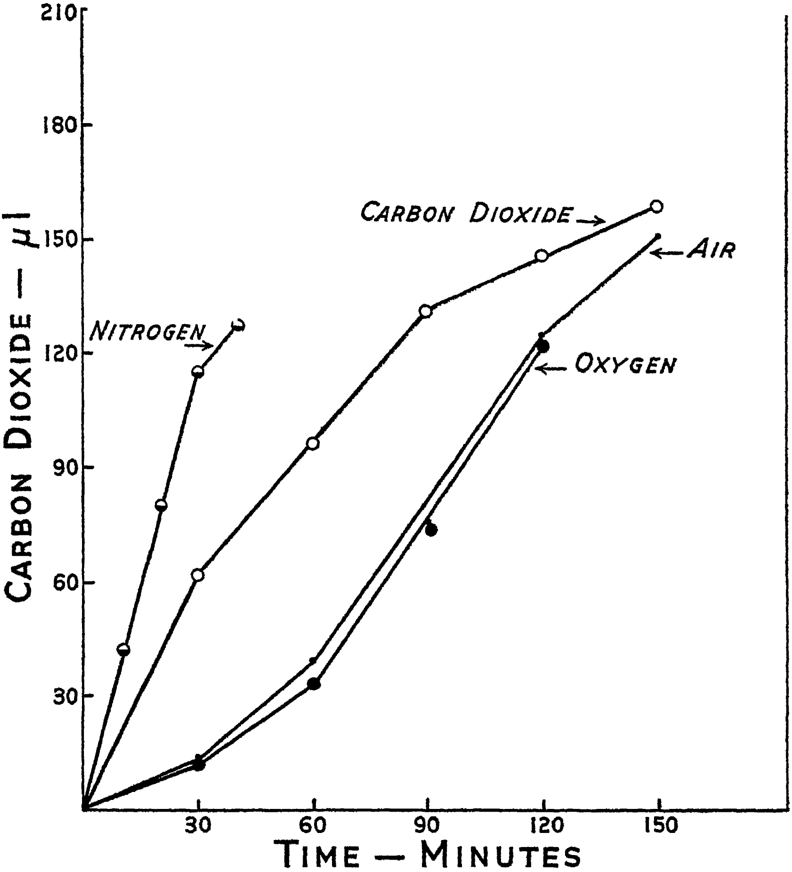


Fig. 2. Carbon dioxide production of flour-yeast water suspensions fermented in air, oxygen, nitrogen, and carbon dioxide.

curves. The carbon dioxide and nitrogen experiments gave about the same gas production in the buffered suspensions and, as might be expected, both were higher than when the fermentation was conducted in air or oxygen.

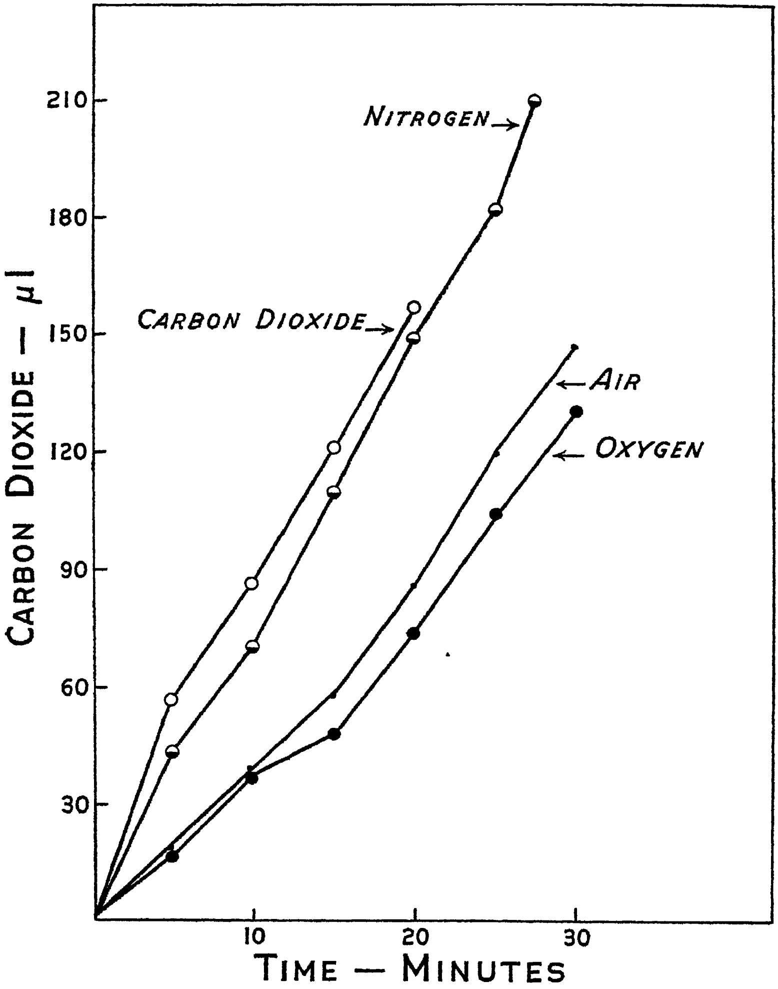


Fig 3 Carbon dioxide production of flour-yeast-buffer (pH 5) suspensions fermented in air, oxygen, nitrogen, and carbon dioxide.

These experiments show that gas production of flour and yeast suspensions is more rapid in an anaerobic atmosphere.

Maltose and Sucrose in the Fermenting Sponges

A sponge was mixed using the ingredients given in the original formula, except that no yeast food was included. After a 5-minute mix the sponge was divided; one-half was fermented in air, the other in a cabinet into which carbon dioxide was conducted. Maltose was determined on 10 g of the sponge by the Blish-Sandstedt procedure, as modified by Sandstedt (1937), at various stages of fermentation. Figure 4 shows that the sponge fermented under carbon dioxide had less reducing sugar at all stages than the sponge fermented in air. The initial increase in maltose around the first hour is always greater in air than in the carbon dioxide-fermented doughs.

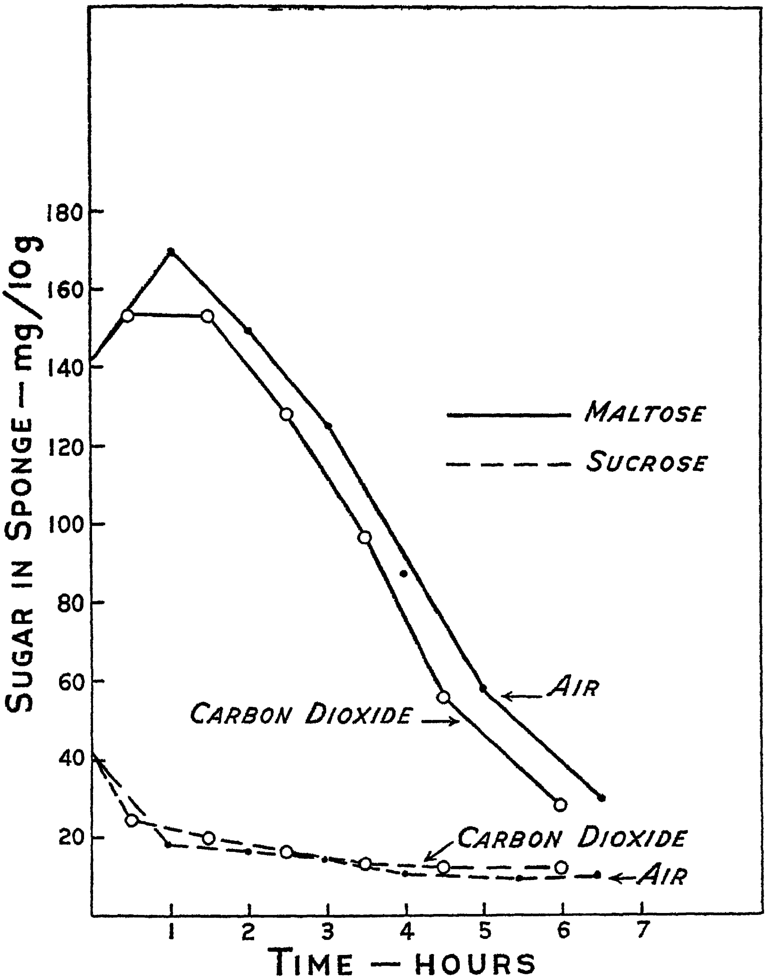


Fig. 4. Maltose and sucrose in sponges fermented in air and carbon dioxide.

Sucrose was likewise determined and its decrease with time is about the same for both doughs.

Retention of Carbon Dioxide in the Cabinet and Open Sponges

As a preliminary check on the amount of carbon dioxide actually contained in the sponges, the following experiment was conducted. Two sponges (39 pounds of flour, 24 pounds of water, 1 pound, 5 ounces of yeast, 1 pound, 15 ounces of shortening, and 0.004% of potassium bromate) were mixed for 5 minutes. The temperature of the sponges was 78°F (25.6°C) from the mixer and 84°F (28.9°C) at maturity. Both cabinet and open sponges were fermented for 4 hours in a room maintained at 80°F (26.7°C) and 85% humidity. At the end of the fermentation period a piece of dough was taken from each sponge. Duplicate 30-g samples from each sponge were weighed in a 250-ml centrifuge bottle and 60 ml 0.1 *N* barium hydroxide solution added. The samples were shaken for 15 minutes in a shaking machine, centrifuged, and 30-ml aliquots titrated with 0.1 *N* HCl solution using phenophthalein as an indicator. The 30-ml aliquots of the open sponge used 26.45 and 26.53 ml of the barium hydroxide solution (30 ml—ml HCl required), while the same size aliquots of the cabinet sponges used 27.40 and 27.22 ml of the barium hydroxide. This proves that the cabinet sponges actually held more carbon dioxide—the equivalent of 0.82 ml of 0 1 *N* barium hydroxide per 15 g of sponge.

Discussion

It is well known that bakers' yeast does not rigidly conform to one metabolic type. Under anaerobic conditions, fermentation is favored; in the presence of air or oxygen, there is an adaptation to the respiratory type. Many organisms, as well as plant and animal tissues, possess both respiratory and fermentation mechanisms and the faculty of using either the aerobic or the anaerobic system, as the conditions require, is known as the Pasteur effect. Much work has been done on the yeast fermentation of glucose under aerobic and anaerobic conditions, but little or no information is available regarding the effect of such conditions on panary fermentation. Fermentation consists of a number of oxidation-reduction reactions of the trioses which are formed by the cleavage of glucose. These reactions depend on several enzyme systems whose delicate balance may be shifted by means of the gaseous environment, or by the presence of certain compounds or groups capable of interference or protection of some of the energy transfer systems. The -SH groups, for instance, undoubtedly have, as one of their functions, the protection of certain systems against oxygen injury and, hence, act as regulators of the fermentation reactions. Before we can understand the reason for some of the overall effects observed in cabinet fermentation, it will be necessary to study certain of these specific enzyme systems. It is quite probable that the atmosphere present above the sponge will influence the rate and the course of many of these reactions since, apparently, the sponge is permeable to the gases above it. This permeability will depend on the rate of diffusion of the individual gases and the efficiency of the sponge in acting as a semipermeable membrane. The velocity of diffusion of a gas is in inverse proportion to the square root of its density. If air is taken as 1, the velocity of diffusion of the other gases is nitrogen, 1.014; oxygen, 0.950; and carbon dioxide, 0.812. However, in the case of a moist membrane in which a gas is soluble, its density exerts a minor effect and the rate of diffusion is proportional to the solubility of the gas in the solvent. Thus, carbon dioxide will pass through a moist membrane more rapidly than oxygen or nitrogen, both of which have a higher velocity of diffusion, because carbon dioxide is appreciably soluble in water. The adverse effect produced by fermenting sponges in an atmosphere higher than normal in oxygen and nitrogen indicates that these gases must diffuse into the sponge. The probability is that carbon dioxide diffuses into the sponge to an even greater extent.

It had been shown manometrically that carbon dioxide production by flour-yeast suspensions is higher in the presence of nitrogen and carbon dioxide, a fact which has been proved numerous times on simpler fermentation systems. That oxygen inhibits true fermentation has been known since the time of Pasteur. Likewise, as further proof, it has been indicated that the total sugar content of sponges fermented in carbon dioxide is less than that of sponges fermented in air. Yet fermentation under nitrogen gives most unsatisfactory results and fermentation under carbon dioxide achieves results better than normal. It is evident that carbon dioxide has a specific effect and this effect is much more important than the maintenance of a state of anaerobiosis. Because of the carbon dioxide produced in the fermentation, anaerobiosis is present, to a large and perhaps sufficient degree, in sponges fermenting in air. The increased amount of carbon dioxide presumably present in the sponges from the cabinet procedure during the period after the break and before the remixing of the sponge may exert its effect by combining chemically with the basic amino groups of the protein, or by changing the course of certain enzyme reactions because of its replacement of air in the fermenting dough. The phenomena of carbon dioxide retention by proteins such as serum is well known. As measured by barium hydroxide titrations, a greater carbon dioxide retention was found in the cabinet sponge than in the open sponge. Until more research is done on the specific mechanism of the action of increased concentrations of carbon dioxide on fermenting sponges, it is not possible to explain fully the effects observed. It is planned to continue this work by studying some of the oxidizing enzyme systems present in dough and the influence of carbon dioxide upon them.

Summary

Comparisons of open and cabinet sponges fermented under controlled conditions of temperature and humidity have shown the marked superiority of the cabinet method. This superiority lies mainly in the production of drier, more mellow doughs that machine more smoothly. From 1 to 2% higher absorption may be employed with the cabinet procedure, and optimum mixing time is always a few minutes shorter than with the conventional open sponge fermentation. Likewise, a shorter sponge time may be employed. In the bread, the main advantages of the cabinet method are a softer texture and thinner cell wall. The effect of cabinets in maintaining some degree of temperature regulation and an optimum humidity in shops not too well controlled for humidity and temperature is obvious.

Numerous gas analyses proved that no significant amount of carbon dioxide is liberated until the sponge breaks; therefore the superiority of the cabinet sponges must be due to the greater concentration of carbon dioxide over the sponge during the period between the breaking of the sponge and the time of remixing. The best results were obtained when the critical concentration of carbon dioxide at this period was approximately 20%. Under commercial conditions, seldom, if ever, are open sponges fermented in an atmosphere containing this high a level of carbon dioxide. However, open sponges will give results similar to the cabinet procedure if carbon dioxide is maintained in the dough room at a high enough concentration.

When sponges are fermented in an atmosphere of either oxygen or nitrogen, poor machining and inferior bread result. This would indicate that the sponge is permeable to the gases above it, and because of the greater solubility of carbon dioxide in water, it is probably more permeable to carbon dioxide than oxygen or nitrogen.

Gas production is more rapid when flour-yeast suspensions are fermented in nitrogen or carbon dioxide than in air or oxygen, but this does not explain the better results obtained with cabinets, since gas production is even more rapid in nitrogen than in carbon dioxide. Neither does any difference in hydrogen ion concentration between open and cabinet sponges account for the results obtained. It is suggested that the greater anaerobiosis obtained by higher concentrations of carbon dioxide is of much less importance than a specific effect of carbon dioxide. This effect may be either a combination of carbon dioxide with basic groups of the protein or the action of carbon dioxide on certain oxidizing enzyme systems present in the fermenting dough, but both possibilities remain to be proved.

Acknowledgments

The authors wish to thank Mr. G. R. Astleford for his valuable help in making the farinograph and extensograph measurements, Mr. Edgar Joseph for the sugar determinations, and Mr. Wendell Buckley for the barium hydroxide titrations.

Literature Cited

American Institute of Baking
1939 Bread Score Report. Am. Inst. Baking, Chicago, Ill.
Baker, J. C., and Mize, M. D.
1937 Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. **14**: 721–734.
Dixon, M.
1943 Manometric Methods. Macmillan, New York, N. Y.
Johnson, H. C.
1945 An investigation of shorter bread sponges. Cereal Chem. **22**: 255–260.
Sandstedt, R. M.
1937 The adaptation of the ferricyanide maltose method to high diastatic flours. Cereal Chem. **14**: 603–604.
Schoonover, F., Freilich, J., and Redfern, S.
1946 Some effects of cabinet fermentation on sponge temperatures and dough and bread characteristics. Cereal Chem. **23**: 186–198.
Umbreit, W. W., Burris, R. H., and Stauffer, J. F.
1945 Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Burgess Publishing Co., Minneapolis, Minn.

FARINOGRAMS AND MIXOGRAMS AS A MEANS OF EVALUATING FLOURS FOR SPECIFIC USES [1]

JOHN A. JOHNSON, J. A. SHELLENBERGER, and C. O. SWANSON

Department of Milling Industry, Kansas Agricultural Experiment Station,
Manhattan, Kansas

(Received for publication January 11, 1946; presented at the Annual Meeting, May 1946)

Many efforts have been made to devise a mechanical means for measuring and recording the properties of doughs when subjected to continuous mixing. At the present time the two machines most popular in the United States for measuring and recording the behavior of dough during mixing are the Brabender Farinograph and the National Micro Recording Dough Mixer or Mixograph. The development of the farinograph and the mixograph and the principles of their action on dough have been thoroughly discussed in monographs describing various physical dough testing methods which have been prepared by Bailey (1940) and Swanson (1943).

The use of recording dough mixers for testing flour quality and flour classification has been studied by several investigators. Brabender (1932) presented farinograms which show the effect of blending North American hard wheat flours with European soft wheat flours. Brabender (1934) also gives experience covering a period of six years in testing baking value of flours by mechanical means. Munz and Brabender (1940, 1941) demonstrated that farinogram patterns have a definite relationship to type of flour. Geddes, Aitken, and Fisher (1940) made an extensive study of the relation between the farinogram characteristics and baking value of Western Canadian wheat. They concluded that the farinogram characteristics were not as valuable as flour protein as an index of loaf volume but the instrument provided valuable accessory information on such properties as absorption, optimum mixing time, and mixing tolerance. Aitken, Fisher, and Anderson (1944) studied the relationship between farinogram measurements and the commercial grade of the wheats from which the flours were milled; the curve dimensions failed to change consistently with decreasing grade.

The wide variation in dough development curves made on the mixograph and the possibilities of classifying these into types were shown by Swanson (1939). Johnson, Swanson, and Bayfield (1943) studied the correlation of mixogram characteristics with baking results. They concluded that the greatest value of such testing was the obtaining of data that supplements baking data. Harris, Sibbitt, and Banasik (1943), employing baking ingredients and fermentation, obtained mixograms whose properties when expressed by a single figure were highly correlated with loaf volume.

---

[1] Contribution No. 125, Department of Milling Industry.

The object of this study was to compare the farinogram and mixogram patterns of flours actually used for specific purposes and to study these patterns in relation to intended types of processing.

Materials and Methods

One hundred and thirty-two flours obtained from flour mills and bakeries located in various parts of the United States and Canada were used. The flours were classified by those supplying the samples. The groups and numbers of samples obtained were as follows: hearth bread (13), bakery (20), topping and doughing (11), family (19), pastry (13), medium quality cake (13), fancy cake (15), specialty flour for pies, cookies, doughnuts, etc. (7), cracker dough (6), cracker sponge (8), and biscuit (7).

The samples, collected over an 8-week period, were stored at 40°F until all were received, at which time they were analyzed for moisture, protein, and ash and the physical dough tests were started. The samples were coded so that during the testing their association with any specific group was unknown to the technician.

Farinograms were made according to the following procedure specified by the Brabender Corporation. The thermostat was maintained at 30°C and the small mixing bowl was used employing 50 g (14% moisture basis) of resifted flour. The absorption for the normal farinogram was determined by a titration curve bringing all doughs to the 500 Brabender unit consistency at point of minimum mobility. Distilled water, only, was employed.

Mixograms were made with the mixograph (National recording dough mixer) operating at 87 rpm with the No. 9 spring setting in an air-conditioned room maintained at 80°F (26.7°C) ± 1°. Thirty-five grams of flour (14% moisture basis) were used with absorptions established from the regression of absorption and protein content previously determined in the laboratory. The farinograph was placed in the same room as the mixograph.

After farinograms and mixograms had been made on all the flour samples, inspection of each group showed considerable variation in curve types, and hence it seemed best to select five farinograms and five mixograms as representatives of each group. The method of selection was by visual inspection of the mixograms and farinograms of each of the groups. First were selected the two curves which represented the extremes in type and the most "typical mean" which was midway between the two extremes. Then two additional curves were selected each of which represented a curve type intermediate between the extremes and the "typical mean."

Many measurements were taken from the farinograms and mixograms but only a few seemed pertinent to this study. Farinogram and mixogram mixing requirement (minutes of mixing to point of minimum mobility), farinogram valorimeter, and mixogram area and absorption determinations were chosen to aid in studying the flours Flour protein and ash content were also included. Statistical means, standard deviations, and coefficients of variability were calculated.

The farinogram and mixogram mixing requirements were determined from the number of curved lines traversed by the pen from the start of the curve to point of minimum mobility. The valorimeter value was obtained according to the method furnished by the Brabender Corporation: The farinogram was placed in a special template (Brabender, 1937) designed to give a single figure strength rating of flours by the farinograms. The template consists of two parts. One part is stationary and has a series of exponential lines which are designated with valorimeter values. The other part is movable and cut to fit the arc made by the pen of the farinograph. The movable part is 12 cm in length. To obtain a valorimeter reading, the farinogram is placed under the stationary part so that the beginning of the curve coincides with both the beginning of the stationary template and the 500 unit consistency line. The movable part is then placed over the stationary part so that the fore edge of the movable template coincides with the point of minimum mobility of the farinogram. The valorimeter value is then read from the stationary template at the point where the opposite edge of the movable template bisects the descending slope of the farinogram. The length of time to point of minimum mobility and the slope after this point determine mainly the valorimeter value. The mixogram area was measured with a planimeter for a mixing period of 8 minutes.

Results and Discussion

The various groups of flours were classified into three broad catagories: (1) bread, (2) pastry, (3) cracker and biscuit flours. The experimental results are thus presented by groups in the three categories by the use of three figures and three tables. The data presented in each of the tables represent the corresponding flours depicted in the figures; in addition, means, standard deviations, and coefficients of variability are given for all flours in each group. The statistical means may not be exactly the same as the "typical means," since the statistical means were obtained by averaging the data from all samples in a group and "typical means" were obtained from curves chosen by observation of general characteristics.

Bread Flours The farinograms and mixograms of the four bread flours are presented in Figure 1. Each set of curves within any one group are arranged from left to right according to increasing strength [2] as indicated by increasing length of mixing requirements, decreasing sensitivity to overmixing, increasing width of band, and general dough development characteristics. The letter under the peak of each curve designates a definite flour. Data with the identifying letters for the corresponding samples are presented in Table I. Neither the farinograms nor mixograms exhibit sharp lines of demarcation between groups but merge gradually from one to another type (Figure 1). The curves for the strongest flours in each respective group tend to be more alike than those for the weakest flours. The curves of the hearth and bakery flours are more uniform than the topping and family flours.

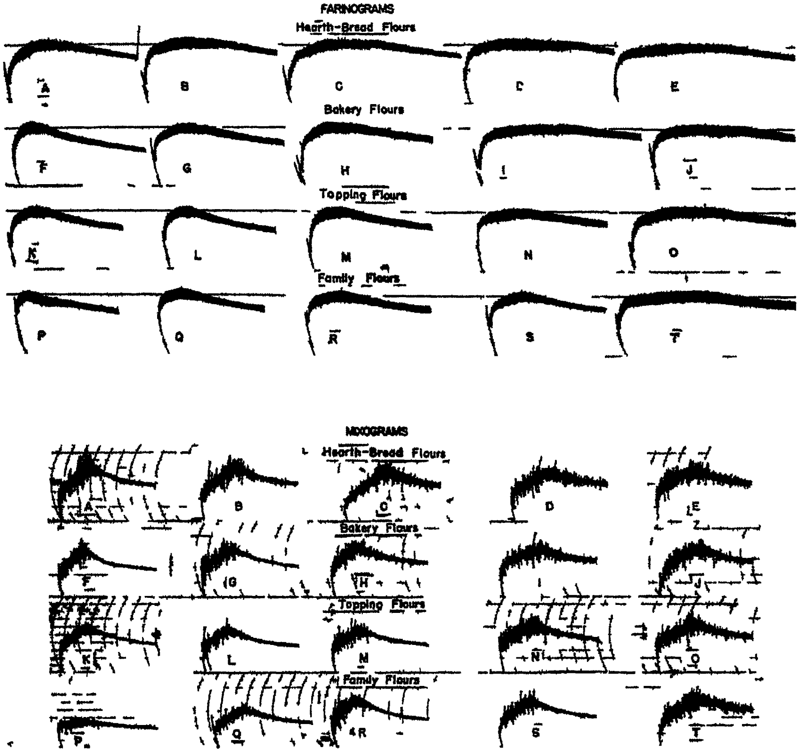


Fig 1 Farinograms and mixograms for bread flours

---

[2] This defines "strength" as used in this paper

TABLE I

SUMMARY OF DATA ON BREAD TYPE FLOURS

Fig. 1 Letter	Protein	Ash	Mixogram mixing time	Farinogram mixing time	Mixogram absorption	Farinograph absorption	Mixogram area	Farinograph valorimeter reading
	%	%	*min.*	*min.*	%	%	*cm²*	*unit*
Hearth flours—13 samples								
A	13.2	.40	2.5	7.5	64.0	64.5	93.6	66
B	13.5	.47	3.0	6.0	64.0	63.5	94.3	70
C	15.2	.48	3.5	8.5	66.0	68.0	85.2	77
D	13.3	.76	3.2	9.0	64.0	65.3	95.0	78
E	14.5	.47	3.2	12.0	65.0	68.9	90.0	83
Mean	13.7	.52	3.20	9.2	64.2	65.8	90.2	75.5
S.D.[1]	.80	.10	.39	1.82	1.03	2.00	6.2	7.07
C.V.[2]	.06	.20	.12	.20	.02	.03	.07	.09
Bakery flours—20 samples								
F	11.3	.44	2.4	3.5	61.0	60.9	71.4	47
G	11.4	.42	2.8	5.5	62.0	63.9	78.6	65
H	12.4	.44	2.2	6.0	63.0	64.9	85.4	65
I	13.5	.45	3.2	9.0	64.0	68.5	90.0	82
J	12.7	.47	3.5	6.0	63.0	64.9	81.9	79
Mean	11.9	.47	3.20	5.6	62.2	63.0	80.7	68.5
S.D.[1]	.94	.04	.72	2.08	1.24	2.18	8.58	12.90
C.V.[2]	.08	.08	.22	.37	.02	.03	.11	.19
Topping flour—11 samples								
K	10.1	.40	2.5	4.5	60.0	62.2	74.9	53
L	10.5	.45	2.7	3.5	60.0	61.2	70.7	45
M	11.4	.48	2.4	5.0	62.0	59.2	73.9	58
N	11.6	.45	3.0	6.5	62.0	63.6	84.8	69
O	12.7	.44	3.2	8.0	61.0	64.7	88.3	82
Mean	11.4	.44	2.90	6.0	62.5	62.3	79.4	63.5
S.D.[1]	1.07	.04	.53	2.11	1.22	2.45	7.25	12.69
C.V.[2]	.09	.10	.18	.35	.02	.04	.09	.20
Family flour—19 samples								
P	9.1	.44	2.0	2.5	59.0	54.9	50.0	38
Q	9.8	.48	2.8	4.5	60.0	58.7	69.2	49
R	11.3	.47	3.0	5.0	63.0	59.5	72.9	52
S	11.0	.43	2.8	6.5	62.0	63.4	85.5	58
T	10.9	.47	3.3	14.0	61.0	59.7	80.6	82
Mean	11.1	.42	2.87	7.1	62.0	62.0	76.4	61.5
S.D.[1]	1.33	.11	.92	3.46	1.73	3.43	10.90	14.80
C.V.[2]	.12	.25	.32	.49	.03	.06	.14	.24

[1] Standard deviation.

[2] Coefficient of variation.

The values in Table I indicate that the protein content had a range of 2% or more in each group. The family flours had slightly greater variation than the hearth, bakery, and topping flours. The hearth flours had the highest protein content with the bakery and topping flours intermediate and the family flours lowest.

Flour absorption is positively correlated with the protein content. Thus, the absorptions for the hearth flours were greater than for the bakery, topping, or family flours. The absorptions determined by the farinograph showed greater variation than those employed in making the mixograms.

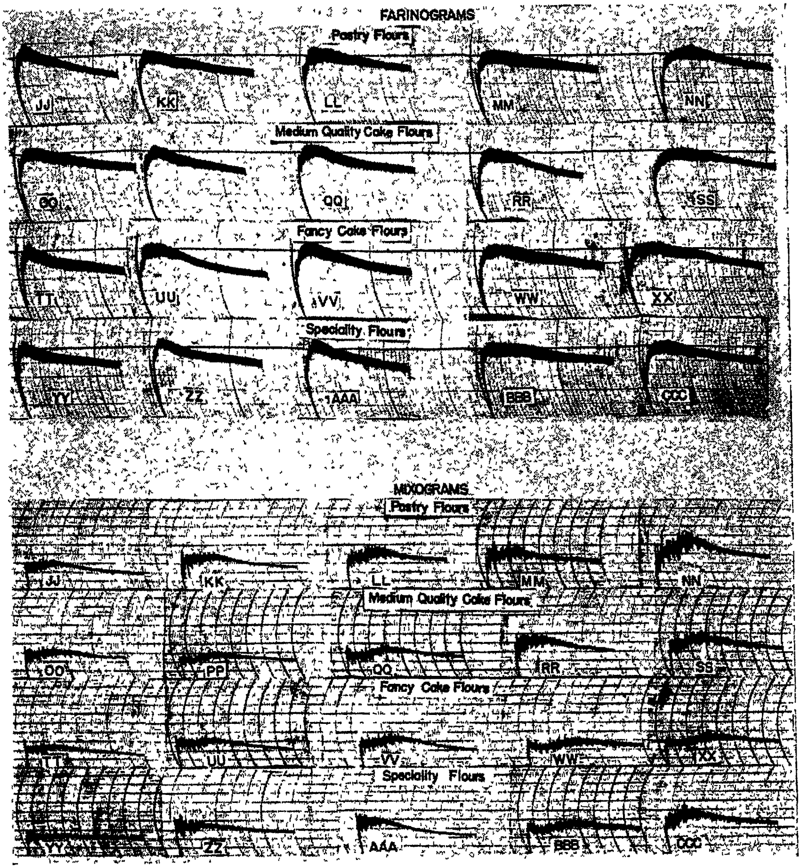


Fig. 2. Farinograms and mixograms for pastry, cake, and specialty flour.

There was only slight variation in the mean mixogram mixing requirement of the four flour groups. The farinograms exhibited greater variation between groups. The hearth group had the longest

TABLE II

Summary of Data on Pastry Type Flours

Fig. 2 Letter	Protein	Ash	Mixogram mixing time	Farinogram mixing time	Mixogram absorption	Farinograph absorption	Mixogram area	Farinograph valorimeter reading
	%	%	*min.*	*min.*	%	%	*cm* ^{2}	*unit*
Pastry flours—13 samples								
JJ	9.1	.41	2.0	2.0	59.0	56.0	49.6	36
KK	7.4	.45	2.2	2.0	56.0	53.9	40.4	40
LL	9.7	.42	2.3	2.5	59.0	55.9	53.7	47
MM	10.0	.54	2.0	1.5	60.0	56.4	58.9	49
NN	9.3	.40	2.5	5.0	59.0	62.3	71.4	54
Mean	9.3	.43	2.2	2.6	58.8	56.7	51.1	43.8
S.D. [1]	1.33	.05	.36	1.43	1.73	2.53	10.08	8.06
C.V. [2]	.14	.11	.16	.55	.03	.05	.20	.18
Medium quality cake flours—13 samples								
OO	8.8	.35	2.3	3.0	58.0	55.1	38.5	45
PP	8.5	.33	2.3	2.0	58.0	52.9	39.4	36
QQ	9.0	.70	2.8	2.0	58.0	52.4	47.7	41
RR	8.9	.43	2.0	3.0	58.0	59.3	51.5	38
SS	10.4	.36	2.8	4.5	60.0	58.0	80.0	53
Mean	8.8	.41	2.3	2.9	57.9	55.2	50.3	41.8
S.D. [1]	.62	.09	.40	.65	.96	3.49	10.39	4.42
C.V. [2]	.07	.22	.18	.22	.02	.06	.21	.11
Fancy cake flours—15 samples								
TT	7.8	.33	2.0	2.0	57.0	51.6	33.7	36
UU	7.9	.36	2.0	3.0	57.0	57.0	40.0	40
VV	8.5	.35	2.2	3.0	58.0	53.6	45.8	43
WW	8.3	.35	3.0	3.5	56.0	53.8	40.0	47
XX	8.8	.39	3.3	4.5	58.0	56.2	49.9	52
Mean	8.6	.37	2.4	3.1	57.8	54.8	45.7	42.1
S.D. [1]	.99	.03	.46	1.86	1.41	2.27	9.98	13.37
C.V. [2]	.12	.08	.19	.60	.02	.04	.22	.32
Specialty flours—7 samples								
YY	7.8	.39	1.2	1.0	57.0	53.5	37.2	38
ZZ	7.0	.44	2.0	1.5	56.0	53.7	48.0	40
AAA	9.2	.44	1.7	1.5	59.0	54.3	52.0	35
BBB	9.8	.42	3.5	4.0	59.0	54.9	48.2	52
CCC	10.4	.43	2.0	3.5	60.0	58.2	53.0	50
Mean	9.6	.41	2.2	2.1	58.1	54.6	47.4	41.6
S.D. [1]	2.40	.03	.79	1.17	1.22	1.63	8.91	7.02
C.V. [2]	.25	.06	.36	.56	.02	.03	.19	.17

[1] Standard deviation.

[2] Coefficient of variation.

mixing requirement and the family, topping, and bakery had shorter mixing requirements. The family flour had the greatest variation in mixing requirement.

The mixogram area and farinogram valorimeter reading were largest for the hearth bread flours, intermediate for the bakery and topping flours, and lowest for the family flours. The within group variation was greatest for the family, intermediate for the bakery and topping, and smallest for the hearth flours. While the four different flour groups are not sharply differentiated by mixogram area and valorimeter reading, the means indicate that hearth flours are the strongest, followed in order by bakery, topping, and family flours.

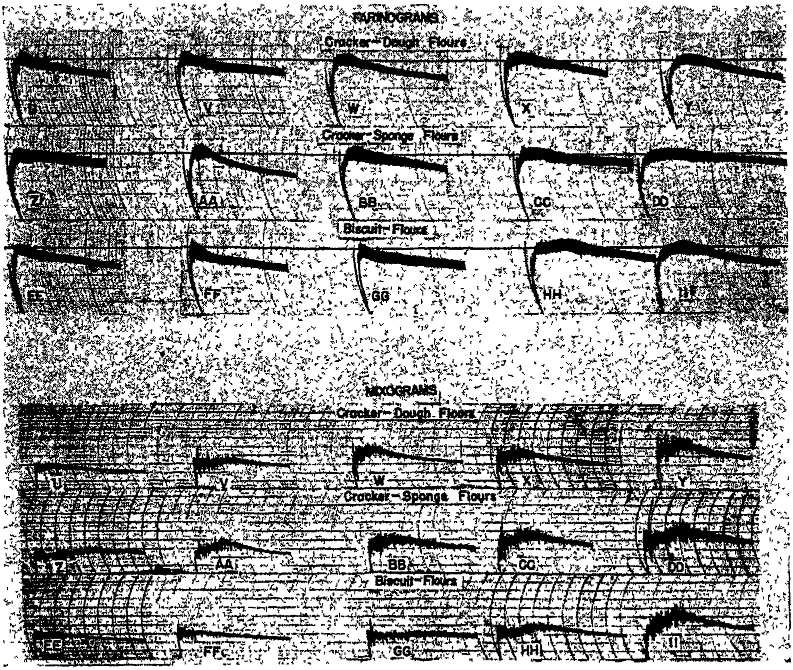


Fig. 3. Farinograms and mixograms for cracker and biscuit flour.

Pastry, Cake, and Specialty Flours. Farinograms and mixograms of flours used for pastry, cake, and several specialties such as doughnut and pie crusts are shown in Figure 2, and the corresponding data are given in Table II. The arrangement of curves and data are the same as used in Figure 1 and Table I. The farinograms and mixograms in Figure 2 are, in general, strikingly different from those in Figure 1. As compared with the bread flours, the pastry and specialty flours are characterized mainly by shorter mixing requirement, greater sensitivity to overmixing, lower protein and ash content, lower absorption, and smaller area and valorimeter reading.

The differences between the four groups of pastry flours (Figure 2, Table II) were small and the variations within each group relatively large, thus making this differentiation into groups of doubtful value. The fancy cake flours have the lowest protein and ash contents, followed by the medium quality cake, pastry, and specialty flours. The specialty flours had the greatest variation in protein content, being followed by pastry, fancy cake, and medium quality cake flours. All

TABLE III

SUMMARY OF DATA ON CRACKER TYPE FLOURS

Fig. 3 Letter	Protein	Ash	Mixogram mixing time	Farinogram mixing time	Mixogram absorption	Farinograph absorption	Mixogram area	Farinograph valorimeter reading
	%	%	*min.*	*min.*	%	%	*cm*^{2}	*unit*
Cracker dough flours—6 samples								
U	9.1	.41	2.1	2.0	59.0	54.5	38.1	40
V	7.4	.38	3.0	1.5	56.0	53.0	41.3	43
W	9.0	.48	2.0	3.0	58.0	59.9	65.0	45
X	9.9	.43	1.8	3.0	59.0	57.4	59.8	47
Y	9.9	.52	2.0	4.5	59.0	61.9	65.3	50
Mean	9.0	.44	2.5	2.8	58.2	56.2	51.2	46.7
S.D.[1]	.93	.05	.85	1.02	1.18	4.45	13.51	5.31
C.V.[2]	.10	.11	.34	.36	.02	.08	.26	.11
Cracker sponge flours—8 samples								
Z	9.3	.39	4.0	2.0	59.0	55.3	42.9	49
AA	9.9	.40	2.5	2.0	50.0	56.9	42.6	39
BB	10.0	.48	2.0	2.5	60.0	57.7	52.4	45
CC	9.9	.40	2.5	3.0	59.0	55.6	60.0	52
DD	10.6	.40	3.2	5.0	60.0	57.7	72.9	62
Mean	9.7	.44	2.4	2.9	59.0	58.3	55.1	47.4
S.D.[1]	1.11	.05	.81	1.09	1.31	3.27	17.29	7.48
C.V.[2]	.11	.11	.34	.38	.02	.06	.31	.16
Biscuit flours—7 samples								
EE	8.2	.44	1.2	2.0	58.0	54.5	42.9	39
FF	7.1	.41	1.9	1.0	56.0	52.2	45.9	32
GG	7.0	.37	3.8	1.0	56.0	51.1	47.1	36
HH	9.4	.36	3.8	6.0	59.0	58.8	60.2	60
II	9.5	.40	2.8	5.0	59.0	61.7	88.6	48
Mean	8.0	.39	2.6	2.4	57.6	54.4	53.9	41.3
S.D.[1]	1.08	.03	1.00	2.15	1.53	4.51	16.31	9.74
C.V.[2]	.14	.07	.39	.90	.03	.08	.30	.24

[1] Standard deviation.

[2] Coefficient of variation.

mixing requirements were short and little difference existed between the four groups. The mixogram absorptions were similar for each group, but the farinogram absorptions for the pastry flours were slightly higher than for the other flours. Only small differences existed between the four groups in the mixogram area and valorimeter reading. The valorimeter readings for the fancy cake flours showed the largest variations.

Cracker and Biscuit Type Flours. Farinograms and mixograms obtained from cracker dough, cracker sponge, and biscuit type flours are shown in Figure 3 and the corresponding data are presented in Table III.

Many of the farinogram and mixogram patterns of the cracker and biscuit type flours were similar to the pastry type flours (Figure 2). The cracker and biscuit flours, in general, exhibited wider variations in mixogram area and valorimeter reading than the pastry type flours. The protein contents and absorptions were about equal for both types, but the cracker flours had slightly larger mixogram area and valorimeter reading. In contrast with the bread type flours, the cracker and biscuit flours had short mixing requirement, lower protein and absorption, and smaller area and valorimeter readings. The ash contents of the cracker flours were about equal to the bread flours.

The differences in curve patterns between groups in Figure 3 and Table III are probably less than the differences within each group. There is no sharp line of demarcation between groups, but the statistical means in Table III show that certain differences exist between groups. The biscuit flours have less protein and ash than the cracker sponge and cracker dough flours. This is likewise reflected in the lower absorption of the biscuit flours. The biscuit flours on the average gave slightly smaller valorimeter readings than the cracker dough and cracker sponge flours.

Conclusions

The farinograms and mixograms presented in Figures 1, 2, and 3 show a wide range in characteristics within groups as well as between groups. This makes it appear that flours used for the same purposes may have widely different mixing characteristics. It is not likely, however, that all flours within a group are equally satisfactory for the purpose intended. On the other hand, it is likely that the many different flours would require different treatment by the baker in order to obtain satisfactory products.

The protein content appears to be one of the most important characteristics determining the farinogram and mixogram patterns. As the protein content increased, the absorption, mixing requirements, mixogram heights, band width, and area and farinogram valorimeter reading also increased. The bread flours which were highest in protein content produced patterns which were distinctly different from those for flours of lower protein content. The bread types are characterized particularly by a long mixing requirement or dough development period. Pastry and cracker type flours, for which low protein is desired, exhibited a wide range of patterns that was generally different from the bread types.

The mixogram area was positively correlated with the valorimeter reading. Both characteristics were positively correlated with protein content. Since protein content, in general, is a factor mainly responsible for strength in commercial flours, it is likely that either the area or the valorimeter reading would give indications of the strength. Strength is desirable for bread flours; while for pastry and cracker types of baking, less strength is desirable. Either the area or valorimeter reading gives figures which indicate in a general way the strength desired for widely different types of baking.

Summary

Farinograms and mixograms were made from 132 commercial flours that were classified into 11 groups on basis of intended usage. The 11 groups were assembled into three categories, namely, bread, pastry, and cracker and biscuit. Protein, ash content, mixing requirements, absorptions, and mixogram areas and farinogram valorimeter readings were employed to study the relationship between curve patterns and intended uses.

Bread flours were characterized by higher protein, higher absorption, longer mixing requirement, and larger mixogram area and farinogram valorimeter readings than the pastry and cracker and biscuit flours. The mixograms of the bread flours had greater height and band width than those made from pastry or cracker and biscuit flours. Cracker and biscuit flours produced curves very similar to the pastry flours and had slightly larger area and valorimeter reading than the pastry flours.

In the bread flours small differences existed between the hearth, bakery, topping, and family groups. In general, as much variation was observed within each group as between the various groups. The hearth group had the highest protein and ash content, longest mixing requirement, highest absorption, and largest mixogram area and valorimeter readings. In these characteristics the hearth group was followed in descending order by bakery, topping, and family flours.

Pastry flours showed little difference, if any, in curve patterns between pastry, medium quality cake, fancy quality cake, and specialty pie crust and doughnut flour groups.

Cracker sponge and cracker dough flours produced patterns that were but slightly different from the biscuit flours, although variation in each group was large. Biscuit flours had shorter mixing requirements, lower absorption, and smaller valorimeter readings than the cracker flours. Little difference was discernible between cracker dough and cracker sponge flours.

Farinograms and mixograms showed a wide range in characteristics within groups as well as between groups. Flours used for the same purpose may have widely different mixing characteristics. It is likely that flours used for the same purpose are satisfactory only if the baker gives each flour different treatment.

Protein content appeared to be one of the most important factors in determining the farinogram and mixogram patterns. Because protein content is mainly responsible for strength in flours and, since mixogram area and valorimeter readings are correlated with protein content, these curve measurements serve to aid in flour classification when fine distinctions between groups or types are not required.

Acknowledgment

The authors wish to thank the Brabender Corporation, Rochelle Park, New Jersey, for loan of their equipment used in this study.

Literature Cited

Aitken, T. R., Fisher, M. H., and Anderson, J. A.
1944 Effect of protein content and grade on farinograms, extensograms, and alveograms. Cereal Chem. 21: 465–488.

Bailey, C. H.
1940 Physical tests of flour quality. Wheat Studies Food Research Inst. 16: 243–300.

Brabender, C. W.
1932 Studies with the farinograph for predicting the most suitable types of American export wheats and flours for mixing with European soft wheats and flours. Cereal Chem. 9: 617–627.
1934 Six years farinography. Cereal Chem. 11: 586–597.
1937 Beitrag zur Weizenstandardisierung. Ein vorschlag fur den Weltweizen-Standard. Foredrag og forhandlinger ved Nordisk Cereal-kjemikerforening og Nordisk. Cerealistforbund Kongress i Oslo (Oslo, 1937), pp. 152–163. Original not seen. From "Physical tests of flour quality," C. H. Bailey, 1940. Wheat Studies Food Research Inst. 16: 268.

Geddes, W. F., Aitken, T. R., and Fisher, M. H.
1940 The relation between the normal farinogram and the baking strength of Western Canadian wheat. Cereal Chem. 17: 528–551.

Harris, R. H., Sibbitt, L. D., and Banasik, O.
1943 The prediction of loaf volume of Hard Red Spring Wheat flours from some properties of mixograms. Cereal Chem. 20: 211–223.

Johnson, J. A., Swanson, C. O., and Bayfield, E. G.
1943 The correlation of mixograms with baking results. Cereal Chem. 20: 625–644.

Munz, E., and Brabender, C. W.
1940 Prediction of baking value from measurements of plasticity and extensibility of dough. I. Influence of mixing and molding treatments upon physical dough properties of typical American wheat varieties. Cereal Chem. 17: 78–100.
1941 American wheat types and varieties as distinguished by farinograms and extensograms. Cereal Chem. 18: 316–337.

Swanson, C. O.
1939 Variations in dough-development curves. Cereal Chem. 16: 625–643.
1943 The Physical Properties of Dough. The Burgess Pub. Co., Minneapolis.

EXTENSOGRAPH STUDIES OF COMMERCIAL FLOURS AND THEIR RELATION TO CERTAIN OTHER PHYSICAL DOUGH TESTS [1]

JOHN A. JOHNSON, J. A. SHELLENBERGER, and C. O. SWANSON

Department of Milling Industry, Agricultural Experiment Station,
Manhattan, Kansas

(Received for publication, December 31, 1945, presented at the Annual Meeting, May 1946)

Interesting relationships between physical dough mixing properties and other quality factors have been disclosed by numerous investigators. However, it is generally believed that testing of doughs under continuous mixing fails to give a complete insight into the effects of such factors as fermentation, mechanical or chemical treatment, on extensibility and related physical dough properties.

Bailey (1940) has presented a comprehensive survey of various physical dough tests, including those made after rest periods. Munz and Brabender (1940) gave a description of the extensograph, an instrument designed to test extensibility and resistance to extension after various periods of rest. They indicate that a study of the rate, direction, and magnitude of change in extensibility and resistance to extension after a time of rest would be a valuable aid in classifying flours for specific uses.

The same authors (Munz and Brabender, 1941) concluded that a combination of farinogram and extensogram data serves to classify soft wheat flours as to their adaptability for specific uses. Soft wheat flours gave extensograms of small area, while the hard wheat flours gave curves of large area and exhibited greater response to rest periods. Pastry and cooky flours exhibited little changes in any extensogram dimension with increasing rest time. Fancy cake flour doughs possessed small extensibility and relatively large resistance to extension as indicated by a large *F/E* ratio. Cracker flour doughs were characterized by larger extensogram dimensions than the pastry, cooky, or cake flour doughs.

Munz and Brabender (1940a and 1941) also demonstrated positive relationships between extensogram dimensions, protein content, and various farinogram measurements. Aitken, Fisher, and Anderson (1944) corroborated the evidence of Munz and Brabender (1941). Significant positive relationships between extensogram length, height, and protein content were found. Farinogram development time was positively correlated with extensogram height.

---

[1] Contribution No. 126, Department of Milling Industry.

The purpose of this investigation was to make a detailed study of the value of extensograms for classifying flours for specific uses. Since data from farinograms and mixograms for the same groups of flours were available, the relationships between extensogram, farinogram, and mixogram characteristics were studied.

Materials and Methods

One hundred and thirty flours obtained from flour mills and bakeries located in various parts of the United States and Canada were used. The following groups and number of flours were obtained: hearth, 13; bakery, 20; topping, 11; family, 19; pastry, 13; medium quality cake, 13; fancy cake, 15; specialty, 8; cracker dough, 6; cracker sponge, 6; and biscuit, 6. A further description of these samples is given by Johnson, Shellenberger, and Swanson (1946).

Extensograms were made according to the instructions supplied by the Brabender Corporation for the use of their instrument. All doughs were mixed in the large farinograph bowl, freed of oxides, to a 500 unit consistency. Flour (300 g, 14% moisture basis), water, and 2% salt were mixed for one minute, followed with a 5 minute rest period and then mixed to the point of minimum mobility. The dough was then scaled to 150 g, rounded up by 20 revolutions of the rounder, molded and placed in the thermostat of the extensograph at 30°C for 45 minutes, and an extensogram made. Repeat tests on the same dough piece were made at 45 minute intervals. For purposes of presentation and discussion, however, only the extensograms made after 45, 135, and 225 minute rest periods were chosen. The extensibility or length (E) was expressed in number of centimeters that the dough was stretched before rupturing. Resistance to extension or height (H) was measured in chart units and represents the maximum force applied to the dough before rupturing. The area was measured with a planimeter and expressed in square centimeters.

The choice of extensograms to represent the various flour groups was made on the basis of maximal, minimal, and "typical mean" values of the curve area. The figures for extensibility and resistance to extension are the values that correspond to the curve chosen on basis of area and may not necessarily correspond to the actual extremes and means of extensibility and resistance to extension. In addition to the curves chosen to represent the flours, the means, standard deviations, and coefficients of variability were calculated from data representing all the flours within given groups. The "typical mean" may not agree exactly with the statistical mean since the former was chosen on the basis of the curve area that approximated the statistical mean.

The farinogram and mixogram data employed to study the relationship of the extensogram characteristics to those of the farinograms and mixograms were, in part, obtained from a previous study reported by Johnson, Shellenberger, and Swanson (1946). In addition to the farinogram and mixogram characteristics discussed by these authors, mixogram heights and protein contents were included in the present study. The maximum value of extensibility, resistance to extension, and area of the extensograms for each flour were correlated with the farinogram, mixogram, and protein content data. The maximum values representing extensibility, resistance to extension, and extensogram area were taken from the extensograms after various rest periods, and thus no uniform rest period is indicated for all extensogram properties nor for the different flours.

Results and Discussion

Extensograms of Flours Intended for Specific Use. Extensograms for flours which gave minimal, average, and maximal curve areas within each flour group are presented in Figure 1. The data for these particular flours are given in Tables I, II, and III, together with the means, standard deviations, and coefficients of variability computed from the results for all flours within each group. The data for the 11 flour groups were assembled into three main categories or types: (1) bread flours, (2) cracker and biscuit flours, and (3) pastry flours.

No sharp line of demarcation may be drawn between the three main types of flours (Figure 1). The extensograms which exhibited maximal areas among the cracker and biscuit flours (curves O, R, and U), are not greatly different from the mean curves E, H, and K for the bread flours. Likewise, the curves of minimum area for the bread flours (curves D, G, and J) are not greatly different from the mean curves (N and Q) for the cracker and biscuit types or the maximal curves (X and Gg) of the pastry flours. However, certain numerical average differences between the three types may be observed. The bread flours (Table I), on the average, contained more protein and produced doughs of greater extensibility, greater resistance to extension and greater extensogram area than either the cracker and biscuit or pastry flour doughs. The cracker and biscuit types (Table II) on the average possessed as much protein as the pastry type flours (Table III). However, the cracker and biscuit doughs exhibited slightly greater extensibility, greater resistance to extension, and greater extensogram area than did the pastry flour doughs. The bread flour doughs exhibited greater response to rest periods than did the other two types. The low extremes illustrated by curves M, P, S, V, and Ee exhibited little response to rest periods compared to most other curves, for example A, B, C, or I.

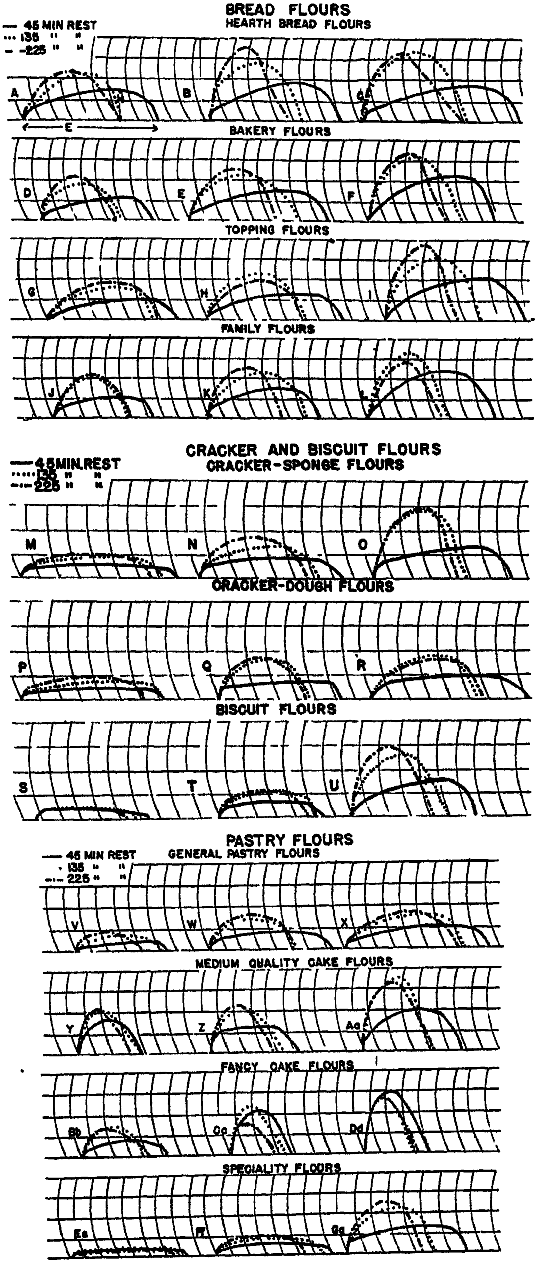


Fig. 1. Extensograms for the various types of flours.

The various groups of bread flours (Figure 1, Table I) tended to have different extensogram properties although no distinct dividing line existed between groups. The mean values for protein and extensogram height and area tended to be highest for the hearth flours, being followed in order by bakery, topping, and family flours. The higher extremes (curves F and I) of bakery and topping flours have properties that are similar to the means for the hearth group. The coeffi-

TABLE I

SUMMARY OF PROTEIN AND EXTENSOGRAM DATA FOR BREAD FLOURS

	Protein	Length			Height			Area		
		45 min.	135 min.	225 min.	45 min.	135 min.	225 min.	45 min.	135 min.	225 min.
	%	*cm*	*cm*	*cm*	*units*	*units*	*units*	*cm*^{2}	*cm*^{2}	*cm*^{2}
HEARTH BREAD FLOURS—13 SAMPLES										
Minimum[1]	11.0	20	15	15	310	470	480	99	105	108
Mean	13.3	26	19	14	320	560	720	113	127	118
Maximum	14.6	27	18	17	345	660	660	125	159	131
Mean[2]	13.7	24	16	15	384	601	649	124	123	119
Std. Dev.	.80	3.7	3.0	3.5	90.9	93.8	74.2	13.9	22.3	26.1
C.V.[3]	.06	.16	.19	.23	.24	.16	.11	.11	.18	.22
BAKERY FLOURS—20 SAMPLES										
Minimum	10.5	26	15	14	235	380	450	62	71	80
Mean	12.4	26	20	17	290	440	515	94	114	106
Maximum	12.6	27	23	28	460	640	680	134	134	114
Mean	11.9	23	18	15	361	563	615	102	114	106
Std. Dev.	.94	2.9	4.9	4.1	68.0	102.2	108.9	18.5	25.9	15.7
C.V.	.08	.12	.27	.27	.19	.17	.18	.18	.23	.15
TOPPING OR DOUGHING FLOURS—11 SAMPLES										
Minimum	10.7	24	21	20	108	365	315	62	98	80
Mean	10.6	26	18	18	260	450	380	85	106	89
Maximum	13.8	25	18	13	425	622	752	138	137	122
Mean	11.4	23	17	15	299	517	515	89	104	94
Std. Dev.	1.07	2.4	2.0	2.4	95.3	115.8	123.1	25.5	13.0	17.8
C.V.	.09	.11	.12	.16	.32	.22	.24	.29	.13	.19
FAMILY FLOURS—19 SAMPLES										
Minimum	10.1	19	15	15	210	415	450	56	75	77
Mean	9.8	21	19	14	305	445	500	87	108	88
Maximum	10.9	23	15	14	490	645	590	146	121	90
Mean	11.1	21	16	15	315	506	518	89	106	93
Std. Dev.	1.33	2.3	2.2	2.7	71.9	111.2	118.8	21.9	21.3	19.8
C.V.	.12	.11	.14	.18	.23	.22	.23	.25	.20	.21

[1] Minimum, mean, and maximum refer to extensograms in Figure 1 for each group.
[2] Statistical mean for each group.
[3] Coefficient of variability for each group.

TABLE II

Summary of Protein and Extensogram Data for Cracker and Biscuit Flours

	Protein	Length			Height			Area		
		45 min.	135 min.	225 min.	45 min.	135 min.	225 min.	45 min.	135 min.	225 min.
	%	*cm*	*cm*	*cm*	*units*	*units*	*units*	*cm*^{2}	*cm*^{2}	*cm*^{2}
CRACKER SPONGE FLOUR—6 SAMPLES										
Minimum[1]	10.7	24	21	20	120	180	200	41	52	54
Mean	9.4	22	18	16	170	270	340	55	63	73
Maximum	10.6	21	14	13	270	560	580	78	90	98
Mean[2]	9.7	24	17	18	158	250	280	56	69	72
Std. Dev.	1.11	1.4	3.2	3.7	47.8	127.1	120.5	12.3	4.4	13.8
C.V.[3]	.11	.06	.19	.21	.30	.51	.43	.22	.06	.19
CRACKER DOUGH FLOUR—6 SAMPLES										
Minimum	9.0	21	21	18	80	150	180	30	44	48
Mean	9.9	19	13	13	125	320	340	40	58	57
Maximum	9.9	24	16	17	200	360	330	68	73	68
Mean	9.0	20	17	16	159	228	258	49	52	52
Std. Dev.	.93	4.8	3.8	2.9	86.2	106.2	91.8	19.2	13.9	10.4
C.V.	.10	.24	.22	.18	.54	.47	.36	.39	.27	.20
BISCUIT FLOUR—6 SAMPLES										
Minimum	7.1	17	15	13	90	90	100	24	20	18
Mean	7.0	15	15	14	140	220	240	32	47	45
Maximum	9.4	19	16	15	310	520	590	79	98	99
Mean	8.0	16	16	15	251	283	324	44	54	66
Std. Dev.	1.08	3.3	2.3	1.5	284	221	230.8	28.5	30.0	45.4
C.V.	.14	.21	.15	.10	1.13	.78	.71	.65	.55	.69

[1] Minimum, mean, and maximum refer to extensograms in Figure 1 for each group.
[2] Statistical mean for each group.
[3] Coefficient of variability for each group.

cient of variability (Table I) for extensibility increased as the length of the curves decreased. This may be caused by the fact that the differences in the flours within a group tended to be accentuated as the rest periods became longer. Most flours in each group tended to produce curves with maximum area at 135 minutes of rest, while with each successive 45 minute rest period, the dough became less extensible and required greater force to be extended.

The cracker dough and sponge flours (Figure 1, Table II) produced doughs with about the same extensibility, but the cracker sponge doughs tended to show more response to 225 minutes of rest than did the cracker dough flour. The biscuit flours produced extensograms of less length and of smaller area than the cracker sponge and dough flours. The low extremes (curves M, P, and S, Figure 1) exhibited little response to rest periods compared to the high extremes (curves O, R, and U). These results appear to corroborate the conclusions of Munz and Brabender (1941).

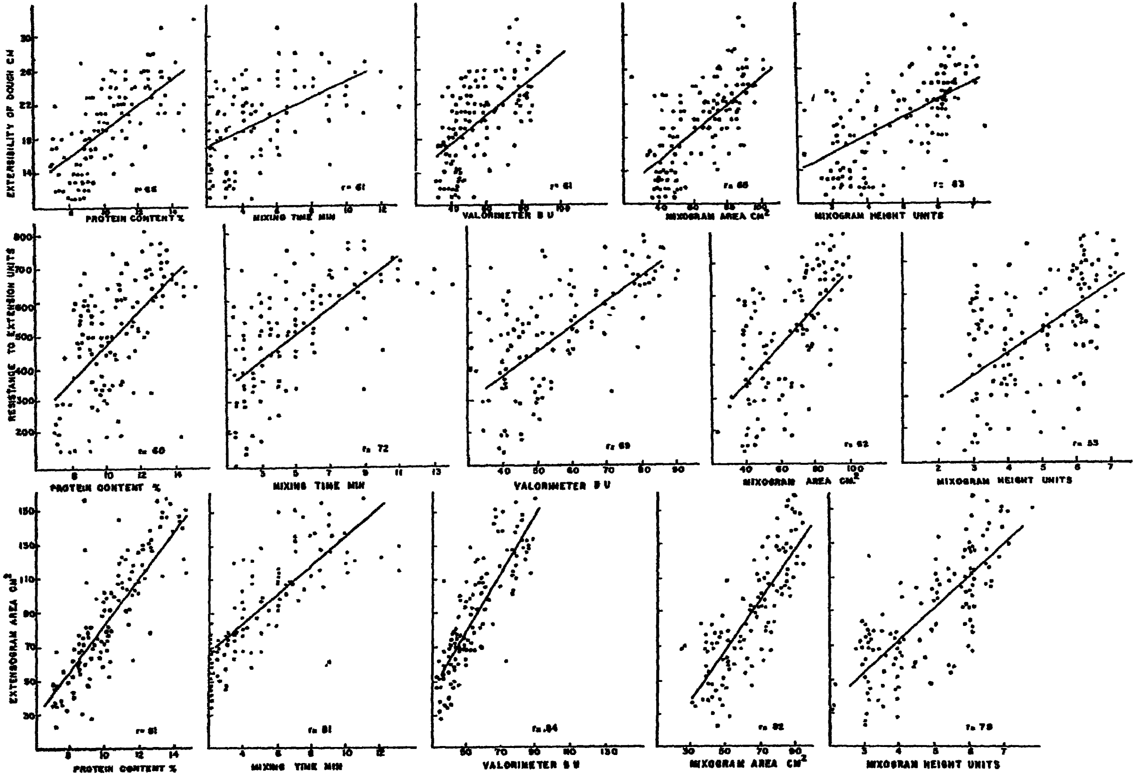
The general pastry flour group (Figure 1, Table III) appears to have characteristics different from those of the cake or specialty flour groups. The general pastry flour doughs are characterized by greater

TABLE III

SUMMARY OF PROTEIN AND EXTENSOGRAM DATA FOR PASTRY, CAKE FLOURS, AND SPECIALTY FLOURS

	Protein	Length			Height			Area		
		45 min	135 min	225 min	45 min.	135 min	225 min	45 min	135 min.	225 min
	%	*cm*	*cm*	*cm*	*units*	*units*	*units*	cm^2	cm^2	cm^2
GENERAL PASTRY FLOUR—13 SAMPLES										
Minimum[1]	7.1	17	15	11	100	170	210	24	37	31
Mean	9.8	21	15	16	170	300	340	55	65	65
Maximum	12.6	25	19	19	200	300	330	69	77	79
Mean[2]	9.3	18	14	14	214	337	352	50	58	60
Std. Dev.	1.33	4.2	4.1	3.8	85.3	103.7	81.5	13.4	13.3	15.0
C.V.[3]	.14	.24	.29	.28	.40	.31	.23	.27	.23	.25
MEDIUM QUALITY CAKE FLOUR—13 SAMPLES										
Minimum	8.5	12	11	9	340	430	440	56	57	48
Mean	8.6	15	12	11	280	440	480	59	69	63
Maximum	10.4	16	11	12	390	750	650	90	101	94
Mean	8.8	14	11	10	339	517	502	67	69	61
Std. Dev.	.62	2.0	1.3	1.4	66.1	114.4	78.8	13.6	19.5	16.3
C.V.	.07	.14	.12	.14	.20	.22	.16	.20	.28	.27
FANCY CAKE FLOUR—15 SAMPLES										
Minimum	7.8	16	11	11	160	300	280	35	42	42
Mean	8.4	11	10	8	425	480	320	59	56	31
Maximum	8.9	11	9	9	590	520	500	80	56	57
Mean	8.6	13	10	10	569	450	391	60	52	47
Std. Dev.	.99	1.7	1.6	1.3	112.9	111.2	72.2	13.5	12.1	93.2
C.V.	.12	.13	.17	.13	.20	.25	.18	.22	.23	.20
SPECIALTY FLOURS—8 SAMPLES										
Minimum	9.2	23	20	19	100	100	70	23	28	24
Mean	8.9	21	19	16	110	170	200	33	48	47
Maximum	9.8	21	15	14	260	460	500	77	90	89
Mean	9.6	20	17	16	138	194	213	40	47	44
Std. Dev.	2.40	2.6	2.6	2.0	62.2	125.0	141.4	20	22.8	22
C.V.	.25	.13	.15	.13	.45	.64	.66	.50	.49	.50

[1] Minimum, mean, and maximum refer to extensograms in Figure 1 for each group.
[2] Statistical mean for each group.
[3] Coefficient of variability for each group.



The relationship of dough extensibility, resistance to extension, and extensogram area to protein content, farinogram mixing time, valorimeter value, mixogram area, and height. The correlation coefficient is included for each relationship.

extensibility and less resistance to extension than the cake flour doughs. The latter exhibit small extensibility in comparison to the resistance to extension. The specialty flour doughs had relatively large extensibility compared to the small resistance to extension. All four groups exhibited wide ranges in all three extensogram properties.

Correlation of Extensogram Properties with Protein Content, Farinogram, and Mixogram Properties. The relationship of dough extensibility, resistance to extension, and extensogram area to protein content, farinogram mixing time, valorimeter value, mixogram area, and height for the 130 samples are shown by scatter diagrams and regression lines in Figure 2. Also, included in the diagrams are the correlation coefficients for each relationship studied.

All the variables studied were positively correlated but the correlations are not of sufficient magnitude to have practical significance for purposes of prediction. Farinogram valorimeter value and mixogram area, which comprise strength ratings obtained with the two mixing devices, respectively, are associated with extensibility and resistance to extension. Furthermore, extensibility and resistance to extension are positively correlated with protein content, which is generally associated with the strength of a flour for breadmaking purposes. Since area of the extensogram is a function of both extensibility and resistance to extension it is to be expected that the area should be related to the same factors with which extensibility and resistance to extension are correlated. Such proves to be the case as shown by Figure 2. The magnitudes of the correlations between extensogram area and protein content, farinogram and mixogram characteristics, respectively, are slightly greater than those for the single extensogram factors.

Summary

Extensograms were made from 130 commercial flours that had been classified into 11 groups on the basis of intended use. The data for the various groups were assembled under three main categories, namely: bread flours, cracker and biscuit flours, and pastry flours. Measurements of extensibility, resistance to extension, and total area were made from the curves obtained after rest periods of 45, 135, and 225 minutes.

No sharp line of demarcation existed between the three types; since each contained flours exhibiting minimal and maximal curve characteristics which tended to merge into those of other groups.

Extensogram data representing bread type flours including hearth, bakery, topping, and family groups showed greater extensibility, greater resistance to extension, and larger curve area than did those from the biscuit and cracker or pastry flours. Bread flour doughs also showed greater response to rest periods than did the other types. The biscuit and cracker group tended to have extensogram properties intermediate in magnitude to the bread and pastry flours. The pastry flours produced extensograms of smallest area.

Protein content, extensogram height, and area tended to average highest for the hearth group being followed in order by bakery, topping, and family flours.

Cracker dough and cracker sponge flours exhibited doughs with about equal extensibility but the cracker sponge doughs had greater resistance to extension and larger extensogram area. The extensograph data for the biscuit flours were extremely variable.

Extensograms for the general pastry and specialty flours were much alike. The extensibility of the dough for these two groups was greater than for the cake flours, while the resistance to extension was less. The fancy cake flour doughs possessed the greatest resistance to extension with 45 minutes of rest, while the medium quality cake flour produced doughs that reached their maximum resistance at 135 minutes of rest.

Extensibility, resistance to extension, and extensogram area were each positively correlated with protein content, farinogram mixing time, valorimeter value, mixogram area, and height. Extensogram properties were more highly correlated with protein content than with farinogram or mixogram properties. The correlations between extensogram area and protein content, farinogram, and mixogram characteristics were greater than the correlations between the same mixing factors and the extensibility and the resistance to extension.

Acknowledgment

The authors wish to thank the Brabender Corporation, Rochelle Park, New Jersey, for the loan of the extensograph used in this investigation. Acknowledgment of the technical assistance of August Schmitz from the Brabender Corporation is also given.

Literature Cited

Aitken, T. R., Fisher, M. H., and Anderson, J. A.
1944 Effect of protein content and grade on farinograms, extensograms, and alveograms. Cereal Chem. 21: 465–488.
Bailey, C. H.
1940 Physical tests of flour quality. Wheat Studies Food Research Inst. 16: 243–300.
Johnson, J. A., Shellenberger, J. A., and Swanson, C. O.
1946 Farinograms and mixograms as a means of evaluating flours for specific uses. 23: 388–399.
Munz, E., and Brabender, C. W.
1940 Prediction of baking value from measurements of plasticity and extensibility of dough. I. Influence of mixing and molding treatments upon physical dough properties of typical American wheat varieties. Cereal Chem. 17: 78–100.
1940a Extensograms as a basis of predicting baking quality and reaction to oxidizing agents. Cereal Chem. 17: 313–332.
1941 American wheat types and varieties as distinguished by farinograms and extensograms. Cereal Chem. 18: 316–337.

AMYLOGRAPH CURVE CHARACTERISTICS OF VARIOUS TYPES OF COMMERCIAL FLOURS AND THEIR RELATION TO FLOUR MALTOSE AND GASSING POWER VALUES [1]

JOHN A. JOHNSON, J. A. SHELLENBERGER, and C. O. SWANSON

Department of Milling Industry, Kansas Agricultural Experiment Station,
Manhattan, Kansas

(Received for publication December 31, 1945, presented at the Annual Meeting May 1946)

Caesar (1932) developed a continuous recording consistometer to measure the changes in consistency of starch dispersions over the entire period of heat gelatinization. Several modifications of this instrument have been developed and used for special studies of the pasting characteristics of starches. Brabender (1937) developed the amylograph which is an improved instrument operating on the same general principle as Caesar's viscosimeter. A full discussion of the working principles of the amylograph is given by Müller (1939). Recently Anker and Geddes (1944) and Brown and Harrel (1944) have thoroughly discussed the operation as well as the theoretical and practical applications of the amylograph to cereal chemical studies.

As pointed out by Anker and Geddes (1944), amylograph curves obtained with wheat flour dispersions represent the result of a number of interacting forces at work as the starch is gelatinized by heat and subjected to amylase action. The peak viscosity of flour pastes depends on several variables such as starch content, particle size, inherent starch characteristics, extent of mechanical injury of the starch, pH, amylase activity, and the rupture of the swollen granules. According to Katz and Hanson (1934), wheat starch swells at 50°C; gelatinization begins at 65°C and is complete at 67.5°C. Amylase action is slight until the gelation point is reached; as the temperature rises above this point, the increase in viscosity due to gelatinization of the starch is opposed by the liquefying action of the amylase and by granule disintegration which results from the internal shearing stress. As more and more of the starch granules become gelatinized and the amylases are heat inactivated, a maximum viscosity is approached at temperatures of 70° to 80°C. The subsequent decline in viscosity is attributed principally to granule disintegration as a result of the internal shearing stress (which becomes pronounced with the increased hydration and closer packing of the granules).

The purpose of this study was to investigate the amylograph curves obtained from a series of widely varying commercial flours manufactured for specific uses. An attempt was made to relate the characteristics of the amylograph curve to the specific uses for which the flours were manufactured and also to interpret the curve characteristics in relation to flour maltose value (diastatic activity) and gassing power.

---

[1] Contribution No. 127, Department of Milling Industry

Materials and Methods

One hundred and thirty flours were obtained from various sources. These flours were classified according to the purpose for which they were used commercially. Eleven different groups were formed which included the following flour types: hearth, bakery, topping, family, general pastry, average quality cake, fancy cake, specialty soft wheat, cracker dough, cracker sponge, and biscuit flours. A more complete description of these samples has been given by Johnson, Shellenberger, and Swanson (1946).

Diastatic activity and gas production values of the flours were determined by the methods described in *Cereal Laboratory Methods* (4th ed., 1941). Amylograph curves were obtained for the entire series of samples.

Operation of the Amylograph. A smooth flour-water slurry was formed by beating and shaking 65 g of hard wheat flour or 55 g of soft wheat flour with 450 ml of distilled water at 30°C. A decrease in the amount of soft wheat flour was necessary because the larger percentage of starch in soft wheat would cause the curve peaks of the amylograms to go above the chart paper.

The suspension was poured into the amylograph bowl, the kymograph adjusted to a zero time position, the contact thermometer adjusted to start heating at 30°C, and the instrument started. The temperature of the suspension was increased at a constant rate of approximately 1.5°C per minute for a total of 40 minutes. After 40 minutes a temperature of 90° C was reached and the amylograph was stopped.

Results and Discussion

The highest and lowest amylograph curves within each group for six of the 11 flour types are shown in Figure 1. Similar results were obtained for the five groups not shown. The dotted line indicates the mean value for all curves within each group.

The curves show large differences in height within the same flour group as well as between groups, and also pronounced differences in the number of minutes required to reach the curve peak. The period of time required before the curves began to rise was uniform within each group. Because of the differences in flour concentration between the bread and pastry groups, there is a difference in the number of minutes required for gelation to start. Any change in the ratio of flour to water will alter the gelation rate and also the curve height.

The amylograph curves used in this study as already stated were obtained from unbuffered water-flour pastes. In some laboratories the pastes are always buffered, but the desirability of buffering may be questioned, especially if the amylograph curves are to be interpreted in terms of starch conversion during the baking of bread. Mangels and Martin (1935) have shown that buffers will influence the diastatic activity differently depending upon whether the same starch type is cooked or raw. Also, the various buffer types, even at the same hydrogen-ion activity, influence the reaction rate of the amylases. Anker and Geddes (1944) studied the effects of two buffer systems on wheat starch over a pH range from 5.2 to 6.7 and observed that the amylograph curve heights were different. Moreover, the amylases are

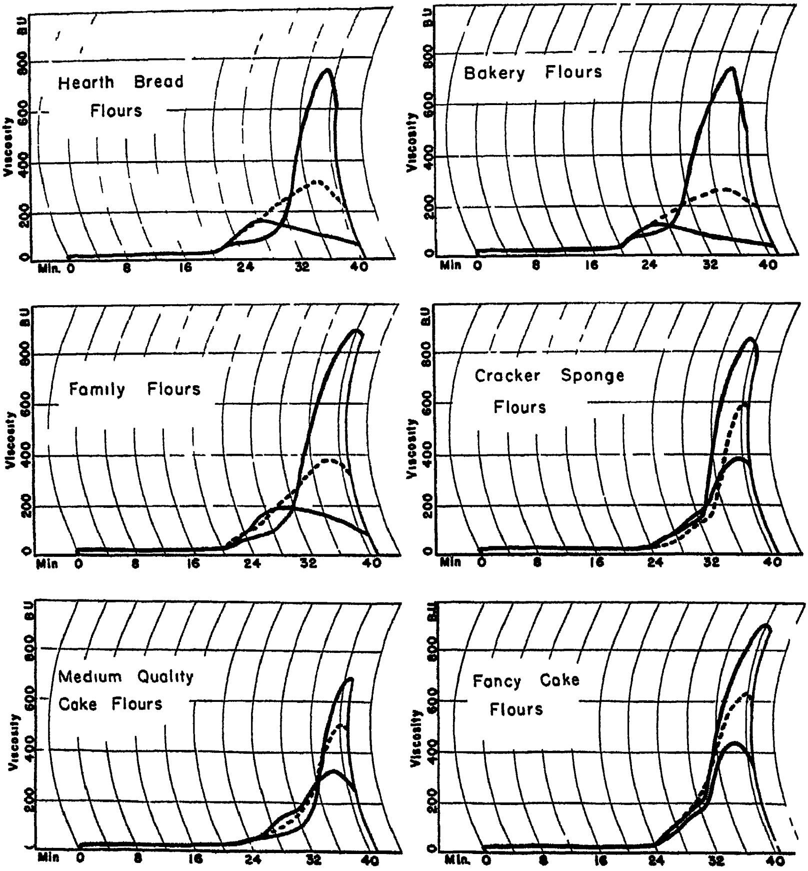


Fig. 1. Highest, lowest, and mean amylograph curves for six types of flours.

known to have different optimum pH values at different temperatures. These considerations complicate the matter of selecting suitable buffer solutions and the proper pH value. Since the change in viscosity recorded by the amylograph is registered only after the starch begins to gel, the pH selected and the buffer adopted should be the ones most effective for the conditions under which the tests are performed. The relationship between amylograph readings and the changes in starch consistency and gas production during the process of baking may be lessened rather than strengthened by the buffering of the amylograph suspensions, since buffers are not added to doughs.

In order to compare the effect of buffering on curve characteristics, a diabasic sodium phosphate-citric acid buffer at a pH of 5.35 was used. It was found that the buffered and unbuffered curves were similar in shape and apparently differed only in height.

Figure 2 shows the relationships between (1) diastatic activity (maltose units — mg/10 g) and gassing power (M.M. mercury pressure at five hours); (2) diastatic activity and amylograph curve height (Brabender units); and (3) gassing power and amylograph curve height.

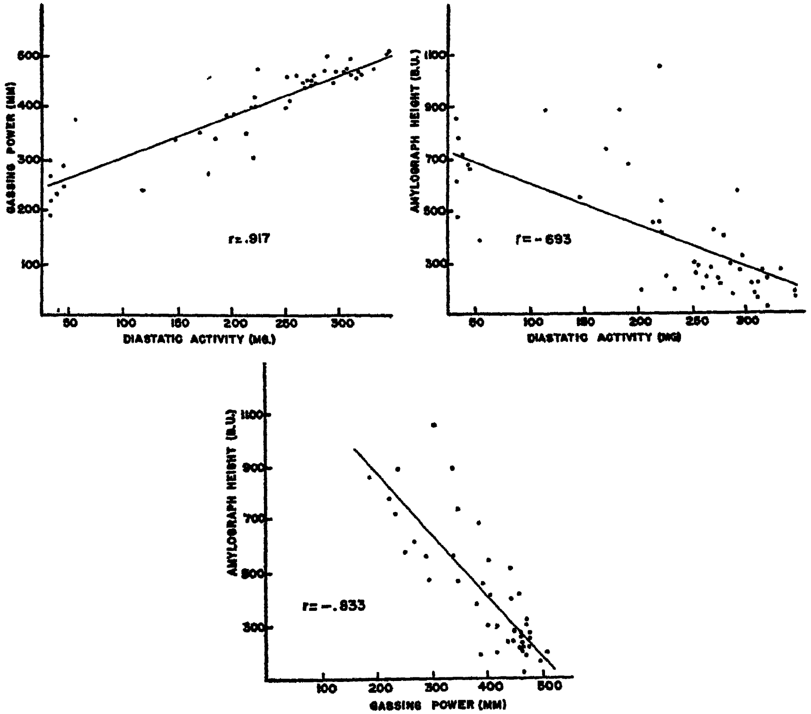


Fig. 2. Relationship between diastatic activity and gassing power, diastatic activity and amylograph curve height, and gassing power and amylograph curve height.

Only the bakery, family, and cracker sponge groups are included in the comparisons since these groups comprise a wide range of flour type. Amylase activity and gassing power are of only minor importance in soft wheat flour.

The relationships between diastatic activity, gassing power, and height of the amylograph curve, as shown in Figure 2, include 45 samples of bakery, family, and cracker sponge type flours. The correlation coefficient for diastatic activity and amylograph curve height was −0.693, for gassing power and amylograph height −0.833, and for diastatic activity and gassing power 0.917.

The relationships among the three measures of amylase activity are interesting. Diastatic activity and gassing power values are obtained from raw starch substrates, one buffered and the other not, while the amylograph readings are made on gelatinized starch at temperatures favoring alpha-amylase activity. The gassing power values involve the use of yeast. Considering the different conditions involved in the measurements, the agreement among the methods is remarkably good.

The variations in the diastatic activity and gassing power values of flours used for the same purpose are well known. The moderate degree of association between these values and the amylograph curve height is indicative that the curves vary considerably within each flour type. The curves did not show any more uniformity within a particular group such as, for example, baker's flour than between flours of entirely different type such as cracker dough and family flour.

Amylographs are being used to some extent by the cereal industry on the theory that the curves give an insight into the changes which starch undergoes within the dough during the baking process. Research to determine more clearly the relationship between the amylograph curves and the changes which doughs undergo during baking is needed.

Another consideration to which thought should be given is the effect of different enzyme types on gelatinized starch during baking. For example, if other amylases than those of malted wheat or barley should be used, the present relationships among the diastatic activity values, gassing power values, and amylograph curves may be altered. Differences in the thermolabilities of amylases from various sources would produce changes in the extent of enzymatic action during baking and be reflected in the amylograph curves.

Summary

Amylograph curves were obtained for 130 samples of commercial flour representing various flour types such as bakery, family, cracker, and cake.

Various types of flour did not give characteristic amylogram curves. Normal curve heights were found to vary nearly as much within a particular flour group as between different flour types.

Amylograph curve heights were negatively correlated with diastatic activity ($r = -0.693$) and with flour gassing power ($r = -0.833$).

Acknowledgment

The authors wish to thank the Brabender Corporation, Rochelle Park, New Jersey, for the loan of the equipment used in this study. They wish also to acknowledge with thanks the technical assistance of August Schmitz.

Literature Cited

Anker, C. A., and Geddes, W. F.
1944 Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21**: 335–360.
Brabender, C. W.
1937 Beiträge zur Strukturforschung von Weizen-und Roggenteigen. Das Mühlenlab. **7**: 121–132.
Brown, R. O., and Harrel, C. G.
1944 The use of the amylograph in the cereal laboratory. Cereal Chem. **21**: 360–369.
Caesar, G. V.
1932 Consistency changes in starch pastes. Ind. Eng. Chem. **24**: 1432–1435.
Cereal Laboratory Methods (4th ed.)
1941 American Association of Cereal Chemists.
Johnson, J. A., Shellenberger, J. A., and Swanson, C. O.
1946 Farinograms and mixograms as a means of evaluating flours for specific uses. Cereal Chem. **23**: 388–399.
Katz, J. R., and Hanson, E. A.
1934 XV Über die scharfe untere Temperaturgrenze der Verkleisterung und ihre Variabilität bei den einzelnen Körenern eines Stärkemusters. Z. physik. Chem. (A) **168**: 321–333.
Mangels, C. E., and Martin, J. J., Jr.
1935 Effect of different buffers and type of substrate on the diastasis of wheat starch. Cereal Chem. **12**: 256–268.
Müller, G. J.
1939 Die Verkleisterungs-Charakteristik der Stärke und ihr Einfluss auf die Qualität von Getreide, Mehl und sonstigen stärkehaltigen Erzeugnissen. Comptes rendus. VI Congres international technique et chimique des industries agricoles, Budapest (Hongrie). **2**: 529–537.

WATER RETENTION CAPACITY AS AN INDEX OF THE LOAF VOLUME POTENTIALITIES AND PROTEIN QUALITY OF HARD RED WINTER WHEATS[1]

KARL F. FINNEY and WILLIAM T. YAMAZAKI[2]

(Received for publication October 4, 1945)

A simple and rapid method for estimating the protein quality of hard wheats is urgently needed, especially so in the early phases of the breeding program where only small samples of grain are available. Such a test has been developed in this laboratory. It consists essentially of a determination of the amount of water held by a flour against centrifugal force applied to a water suspension to which lactic acid has been added. The amount of water so held is referred to herein as the water retention capacity. This paper describes the test and presents data obtained in its application to hard winter wheats.

Development and Theory of the Test

Viscosity as a measure of the imbibitional capacity of flour has been advanced by Sharp and Gortner (1923) as an index of flour strength and is extensively used in evaluating soft winter wheats. It has not been applied successfully, however, to varieties of hard wheat. The senior author has determined the viscosity at several protein levels for each of six varieties of hard red winter wheat—Kharkof, Blackhull, Tenmarq, Pawnee, Comanche, and Chiefkan.[3] The relation between viscosity and protein content for three of these varieties is shown in Figure 1A. It appears from these data that viscosity is a linear function of the *quantity of protein* within a variety and that the regression of viscosity on protein content is different for each variety. However, viscosity does not properly evaluate the three varieties since, on the basis of loaf volume–protein relations, Kharkof and Tenmarq have approximately equal protein quality and both are far superior to Chiefkan; whereas, on the basis of the viscosity–protein relations, Tenmarq is definitely inferior to Kharkof and nearly as poor as Chiefkan.

The fact that viscosity does not increase with protein content in the same manner and to the same degree as does loaf volume appears to be due to some physical property of the protein such as the ease with which it is hydrated. It was thought that by taking viscosity and some function of rate of hydration, such as mixing time, into account a better measure of protein quality might be derived. Accordingly, after preliminary trials, viscosity was multiplied by the square root of the mixing requirement of each variety sample as determined by the mixograph. The relation between protein content and viscosity corrected in this manner is shown in Figure 1B. It will be

---

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associate Chemist and Junior Chemist, respectively, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

[3] Paper presented at the A.A.C.C. tri-section meeting, Manhattan, Kansas, 1941.

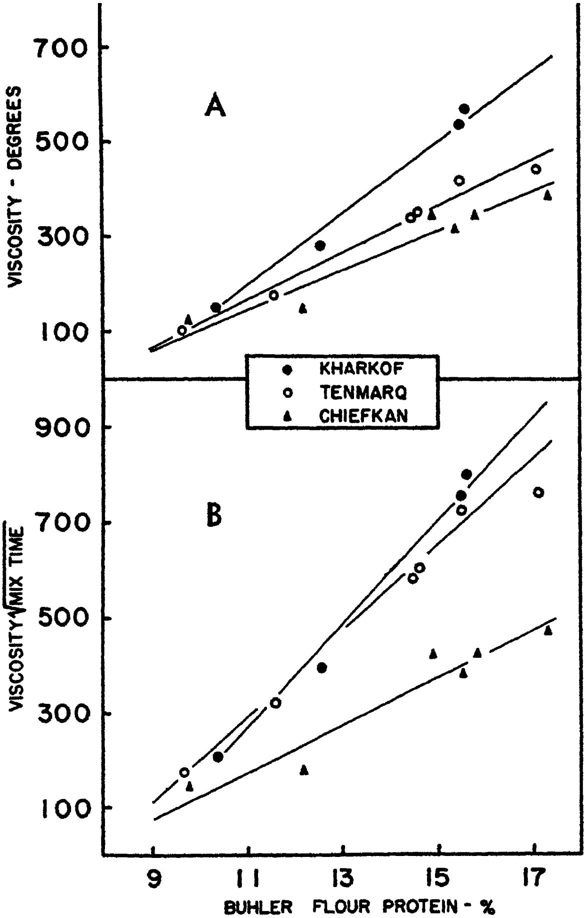


Fig. 1. Relation between protein content and viscosity (above), and viscosity corrected by a hydration factor (below) for Buhler-milled flours of Kharkof, Tenmarq, and Chiefkan.

noted that the regression lines for Kharkof and Tenmarq are now similar, indicating about equal quality, and that both are far above Chiefkan.

Further consideration suggested that the hydration factor possibly was indirectly measuring some physical property such as the tenacity with which the protein holds water in acid media and/or its relative impermeability under certain conditions of acidity and pressure. It seemed reasonable to expect that an easily hydrated protein meshwork might hold its water of hydration less firmly or might be more permeable than one which is hydrated with more difficulty. There appeared to be a logical analogy between this concept of the capacity of flour proteins to imbibe and to hold water against pressure and certain facts known about gas production and retention in the baking process. For example, in a study with 36 hard winter flours varying in protein content from 9 to 18% for each of six varieties of varying protein quality, proof height in a given time was linear and very highly correlated with protein content ($r = 0.97$). However, when gas production and expansion were greatly increased at the elevated temperature of the baking oven, enormous differences between varieties in gas-retaining ability of the protein were apparent as is indicated by the comparatively low correlation coefficient ($r = 0.81$) for loaf volume and protein content of the same samples. These correlation coefficients indicate that inherent differences in gas-retaining capacity of flours differing in protein quality were not manifested until the protein had been sufficiently inflated and expanded by the gas pressure created in the baking oven. Accordingly, it was conceived that this pressure created in the oven might be paralleled in lactic acid-flour-water suspensions by applying centrifugal force to the water-inflated protein and thereby obtain a measure of water retention capacity for correlating with gas retention capacity.

Material and Methods

The wheat used in this study consisted of representative samples of varieties of hard red winter wheat grown at various locations in the Great Plains area as a part of the coordinated wheat improvement program carried out by the U. S. Department of Agriculture in cooperation with the state agricultural experiment stations. A study was first made of 51 samples of flour milled on the Buhler experimental mill and later of flour produced by grinding wheat in a Hobart Coffee Mill and separating the bran and flour by sifting. The purpose of this latter study was to determine to what extent flour from small samples such as could be ground in a Coffee Mill would provide reliable results. The samples milled on the Buhler mill were from 10 varieties of varying protein quality, Kharkof, Blackhull, Tenmarq, Cheyenne, Nebred, Pawnee, Comanche, Chiefkan, Red Chief, and Wichita, each of which was represented by a low, medium, and high protein level for each of the crop years 1941 and 1942, except for the last two varieties which were from the 1942 crop only. The Hobart flours were milled from the same lots of Chiefkan, Tenmarq, Comanche, and Cheyenne mentioned above and from three additional lots of Chiefkan and one of each of the other three varieties from the 1943 crop. Thus, 23 of the 29 Hobart flours were from the same samples of wheat milled on the Buhler.

The Buhler mill was operated as described by McCluggage, Anderson, and Larmour (1939). Previous to milling on the Hobart grinder, each 75.0 g wheat sample was passed through the rolls of the Tag-Heppenstall moisture meter. On the basis of the wheat moisture, each Tag-broken sample was tempered to 14% moisture for 24 to 48 hours, after which the Hobart flours were obtained after two grindings on the Hobart Coffee Mill and two siftings. The extremely simple flow sheet showing wire sieves (meshes per inch) and grinder settings (No. 1 and No. 1 – or closed) is shown in Figure 2. The burrs of the grinder touched very faintly at a setting of 1¼. The average total time required for grinding, sifting, weighing, and labeling products, calculating yield, and cleaning out the grinder for the next sample averaged 11 to 12 minutes.

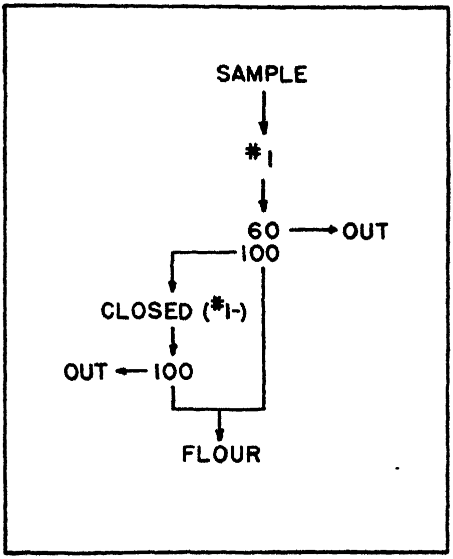


Fig. 2. Flow sheet for Hobart milling procedure showing wire sieves (meshes per inch) and grinder settings (No. 1 and No. 1 –).

The determination of water retention capacity consisted, essentially, of centrifuging a small sample of flour after soaking in water in a test tube to which lactic acid had been added. After centrifuging and pouring off the supernatant liquid, water retention capacity was determined from the increase in weight and expressed as a percentage of the flour.

Preliminary studies showed that the differentiation between varieties increased with the quantity of water used for the soaking and leaching operation previous to adding the lactic acid. In general, however, it is impractical in making numerous determinations to use test tubes larger than about 100 ml. Accordingly, as large a quantity of water was used as was practical with a 100-ml test tube Studies in varying the quantity of concentrated lactic acid indicated that the ratio of total water to lactic acid should be about 4 to 1 for Hobart flours averaging about 0.90% ash. Lower concentrations decreased differentiation and higher concentrations produced distorted results. With Buhler experimentally milled flours averaging 0.45% ash, however, a satisfactory ratio of total water to concentrated lactic acid was about 67 to 1. The considerably smaller quantity of lactic acid used for Buhler compared to Hobart flour indicates the consideration which must be given to ash content. Studies with the time and speed of centrifuging indicated that 25 minutes at 1800 rpm was satisfactory

The details of the procedure used were as follows 5 g of flour was weighed into previously tared, rubber-stoppered, 100-ml pyrex test tubes. Eight determinations were carried out simultaneously. For Hobart flour, 60 ml of water was added to each of the eight tubes one at a time. Immediately after each addition, the stoppered tube with its contents was shaken vigorously for a few seconds to completely wet and suspend the flour. After three additional shakings and a rest period of 4 minutes for sufficiently complete hydration, the stoppers were removed and 20 ml of lactic acid (four parts 85% acid and one part water) was added to each. The eight 20 ml portions were added simultaneously from a unit of eight smaller test tubes. After allowing 1 minute for draining, each was mixed by gently teetering immediately after stoppering. After three more gentle mixings and a rest period of 4 minutes, each stopper was removed and scraped clean at the tube opening. The tubes and contents were then centrifuged for 25 minutes at 1800 rpm in an International Centrifuge, size 2, the supernatant liquid was gently but quickly decanted off, and the tubes allowed to drain into wads of cotton for 5 minutes at an angle of 15° to 20°, after which any liquid adhering at the tube opening was swabbed off with the cotton. The tubes were then stoppered and weighed.

The same procedure was followed for Buhler experimentally milled flours, except that 80 ml of water and 1.5 ml of lactic acid (four parts concentrated and one part water) were used instead of 60 and 20, respectively. Forty-eight determinations can be carried out by one person during an 8-hour day.

The Buhler experimentally milled and unbleached flours were baked using the rich, highly bromated, milk-containing formula in conjunction with optimum mixing time as described by Finney and Barmore (1945, 1945a). The standard deviation for the average of duplicate determinations made in this laboratory on different days is 10 to 13 cc for loaf volume and 0.75 to 1% for water retention capacity.

Experimental Results

The relation between water retention capacity and loaf volume of bread was first determined for the 51 Buhler experimentally milled flours. The results, with and without lactic acid, are shown graphically in Figure 3. With no lactic acid, there is obviously no relation between the quantity of water retained and loaf volume. With lactic acid, however, there is a high positive correlation ($r = 0.95$) and the relation is obviously linear. The standard error of estimate is 55 cc. Considering the large amount of differentiation between samples of over 700 cc, it appears that water retention capacity of the Buhler-milled flours is an excellent index of their loaf volume potentialities.

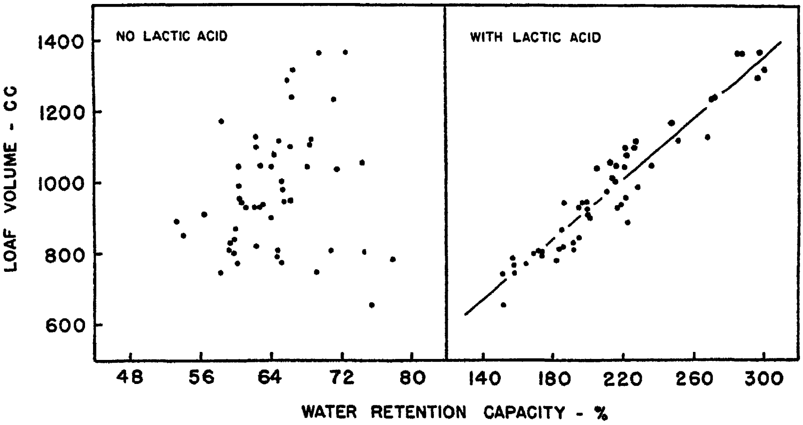


Fig. 3. Relation between loaf volume and water retention capacity with and without lactic acid for 51 Buhler experimentally milled flours representing two crop years and several protein levels for each of 10 hard winter wheat varieties of varying protein quality.

There were six Chiefkan and three Red Chief samples in the group of 51 Buhler-milled flours. Since the protein quality of these two varieties is generally considered to be distinctly poorer than any of the other eight represented in this study, a pertinent question relates to the relative abilities of water retention capacity and protein content to predict loaf volume. The correlation of protein content with each of the two factors loaf volume and water retention capacity gave coefficients of $r = 0.89$ and $r = 0.84$, respectively, as compared with $r = 0.95$ for water retention capacity and loaf volume. The partial correlation between water retention capacity and loaf volume independent of protein content gave a coefficient of $r = 0.82$, indicating that water retention and loaf volume are very highly correlated within groups of samples having the same protein content. The correlation coefficient of $r = 0.95$ for water retention capacity and loaf volume is only slightly less than the multiple correlation coefficient of $R = 0.965$ whereby protein content is taken into account. The standard partial regression coefficients of $\beta = 0\ 686$ for loaf volume on water retention capacity independent of protein content and of $\beta = 0.314$ for loaf volume on protein content independent of water retention capacity indicate that water retention capacity is more than twice as effective in forecasting loaf volume as is protein content. Nevertheless, the smaller of the two betas is significant far beyond the 0.1% point. Thus, the various statistical calculations indicate that water retention capacity is an expression of both the quality and quantity of protein.

Water retention capacities and protein contents for the Hobart-milled flours are presented in Table I, together with protein contents and loaf volumes for the 23 Buhler flours.

It will be noted that the protein contents of the Hobart flours varied from 0.7% to 2.2% more than the corresponding Buhler experimentally milled flours, except for one which was 0.6% less. The Hobart flours averaged about 1% higher in protein than the Buhler-milled flours. Accordingly the "as received" water retention values were corrected with the aid of the data in Table II (presented later for convenience) to those values expected for Hobart flours having 1% more protein than the corresponding Buhler flours, thereby making the two sets of data comparable. The protein-corrected water retention capacities are included in Table I (last column) and are plotted against the loaf volume for Buhler flours in Figure 4. The correlation coefficient is nearly the same ($r = 0.94$) as was obtained for water retention capacity and loaf volume of the Buhler flours, and the regression is likewise obviously linear. The standard error of estimating loaf volume from Hobart flour water retention capacity is 60 cc. The number of samples is too small to permit positive conclusions, but the results indicate that water retention capacity of Hobart-milled flour is a useful index of loaf volume.

The partial and multiple correlations involving the data for the Hobart flours gave results very similar to those for the Buhler flours, and indicated that water retention capacities for Hobart and loaf volumes for Buhler milled-flours are very highly correlated within groups of samples having the same protein content, that water retention capacity is more than 2½ times as effective in predicting loaf volume as is protein content, and that water retention capacity is an expression of both the quality and quantity of protein.

TABLE I

Protein Content and Physico-Chemical Data for Buhler- and Hobart-Milled Flours of 29 Hard Red Winter Wheats Grown under Different Environments and Representing Three Crop Years and Several Protein Levels for Each of Four Varieties of Varying Protein Quality

Variety and location	Crop year	Buhler flour[1]		Hobart flour[1]		
		Protein[2]	Loaf volume	Protein[2]	Water retention cap.	
					As rec'd	Adjusted for prot.[3]
		%	%	%	%	%
Tenmarq						
N. Platte, Neb.	1941	13.8	1048	15.2	154.8	151.6
Manhattan, Kans.	1941	12.4	938	13.5	162.5	161.7
Amarillo, Texas	1941	10.0	768	10.9	129.6	130.4
Hays, Kans.	1942	17.1	1235	17.9	197.0	198.6
N. Platte, Neb.	1942	15.6	1123	15.0	150.3	163.1
Woodward, Okla.	1942	10.9	830	11.9	143.2	143.2
Manhattan, Kans.	1943	—	—	11.4	135.9	—
Comanche						
Woodward, Okla.	1941	13.1	928	14.1	148.8	148.8
Amarillo, Texas	1941	11.1	820	12.2	140.8	139.8
Hays, Kans.	1942	18.0	1368	18.7	219.8	222.9
N. Platte, Neb.	1942	14.4	1045	16.2	170.1	161.9
Woodward, Okla.	1942	11.0	825	11.9	147.6	148.6
Manhattan, Kans.	1943	—	—	13.6	170.1	—
Cheyenne						
Sheridan, Wyo.	1941	14.7	927	16.1	166.8	163.5
Lincoln, Neb.	1941	12.8	929	14.0	147.8	146.1
Manhattan, Kans.	1941	11.5	810	12.3	146.0	147.7
Amarillo, Texas	1941	9.5	733	10.4	125.8	126.6
Hays, Kans.	1942	16.9	1120	18.0	195.9	195.1
N. Platte, Neb.	1942	12.9	948	15.1	157.0	147.0
Manhattan, Kans.	1943	—	—	12.6	142.1	—
Chiefkan						
Lawton, Okla.	1941	15.3	921	16.5	148.1	146.8
Lincoln, Neb.	1941	13.2	783	14.2	135.6	135.6
Amarillo, Texas	1941	10.6	655	11.5	116.7	117.3
Hays, Kans.	1942	17.1	1037	17.8	165.8	167.8
N. Platte, Neb.	1942	12.9	748	14.4	129.4	126.1
Woodward, Okla.	1942	11.7	793	12.6	132.4	133.0
Manhattan, Kans.	1943	—	—	13.6	136.7	—
Manhattan, Kans.	1943	—	—	13.2	129.9	—
Manhattan, Kans.	1943	—	—	13.1	133.2	—

---

[1] The average ash contents for the Buhler and Hobart milled flours were 0.48% and 0.84%, respectively.

[2] 14% moisture basis.

[3] The "as received" water retention values were corrected with the aid of the data in Table II to those values expected for Hobart flours having 1% more protein than the corresponding Buhler flours.

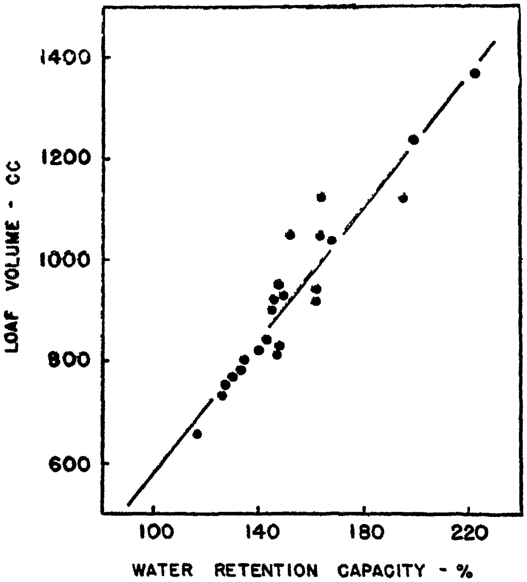


Fig. 4. Relation between Buhler flour loaf volume and Hobart flour water retention capacity for 23 hard red winter wheats representing two crop years and several protein levels for each of four varieties of varying protein quality (each water retention capacity has been corrected to a protein content 1% greater than that of the corresponding Buhler-milled flour by means of Table II).

In the practical application of the water retention capacity test, however, it must be considered in relation to the effect of variety and protein content. Accordingly, the "as received" water retention values and protein contents for the Hobart flours are plotted in Figure 5 for each of the four varieties (Table I). Pertinent statistical data are given in Table II. These data indicate that water retention capacity is a linear function of protein content within a variety.

For convenience in making comparisons, the regression lines of Figure 5 are repeated in Figure 6. These regression lines and their slopes (Table II) suggest a family of lines which tend to fan out from a common origin such that at a given protein content the slopes for the

TABLE II

Correlation Coefficients and Regression Equations for Water Retention Capacity and Protein Content of the Hobart Flours Representing Four Hard Winter Wheat Varieties (x = Protein Content)

Variety	Correlation coefficient r	Regression equation
Comanche	0.91	10.30x + 16.55
Tenmarq	0.91	8.02x + 42.78
Cheyenne	0.96	8.36x + 36.00
Chiefkan	0.93	6.56x + 43.30

variety regression lines are positively correlated with water retention level (protein quality). These slopes are not different statistically, but a similar relation appears to exist for loaf volume and flour protein content (Finney, 1943) and has been established statistically for baking absorption and flour protein content (Finney, 1945). Thus, it appears justifiable to use the regression lines of Figure 6 as a basis for a correction chart representing all probable water retention capacity (protein quality) levels. Such a chart can be constructed by drawing in regression lines between and beyond those for Comanche and Chiefkan. By correcting the lactic acid water retention capacities of the Hobart flours obtained from new wheat varieties to a constant protein basis such as 14½% (average for Hobart flours), an estimation of their protein quality or bread-making capacity relative to that for known varieties can be made quickly. As an example of the application of Figure 6 in making corrections for protein in variety evaluation, consider the data given in Table I for the last Cheyenne sample from Manhattan as representing a variety of unknown protein quality. Its "as received" water retention value of 142.1 at a protein content of

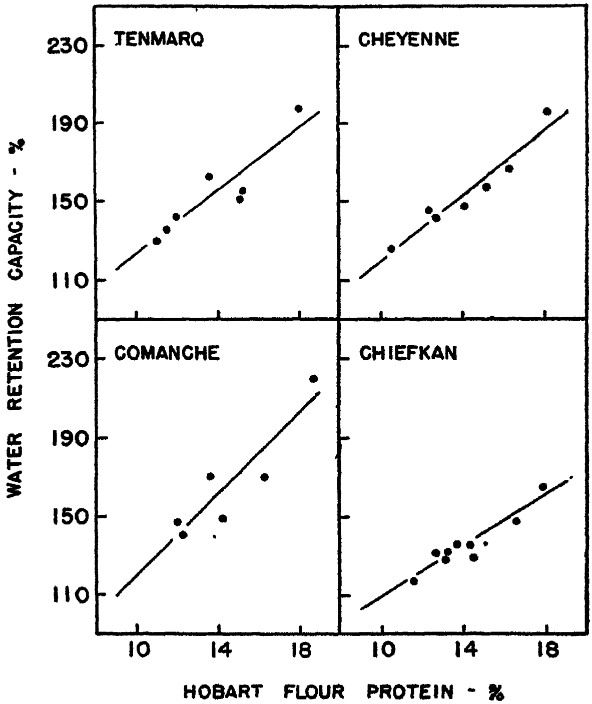


Fig. 5. Relation between water retention capacity and protein content of flours milled on a Hobart grinder from 29 hard red winter wheats representing four varieties of varying protein quality.

12.6% falls approximately on the Cheyenne regression line, thereby classifying the flour as being equal to Cheyenne in protein quality. The value of 142.1% retention is increased to 157% by following up the Cheyenne regression line to 14½% protein, at which point the water retention values of known and experimental varieties might be compared with a minimum of correction when considering all samples.

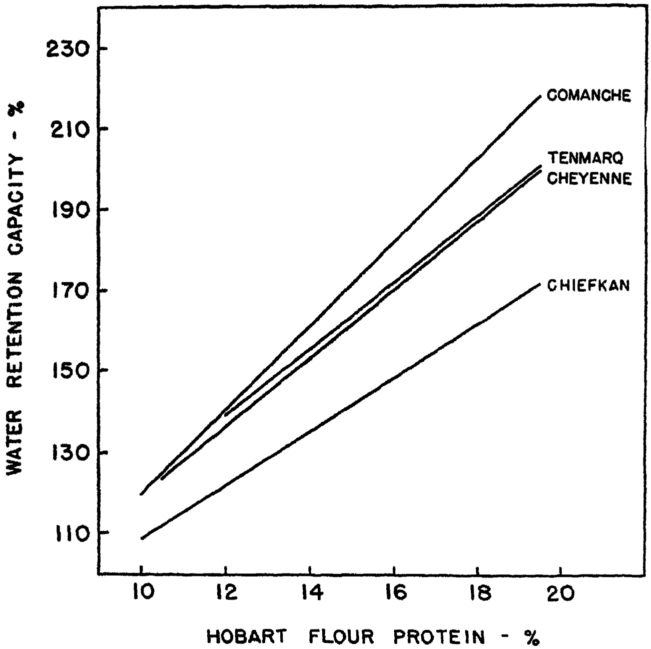


Fig 6. Water retention capacity–protein content regression lines calculated from the Hobart flour data plotted in Figure 5

The water retention capacity test appears to have excellent possibilities of being particularly valuable in wheat breeding where the limited amounts of material in the early phases of the program are insufficient for the usual milling and baking procedures. The test on Hobart flours also has possibilities in the inspection and purchasing of wheat in territories where undesirable varieties are grown.

Summary

Water retention capacity and loaf volume data are reported for samples of hard winter wheat varieties covering a wide range in protein content derived from a very wide range of climatic and soil conditions, and representing three crop years.

Water remaining imbibed after the application of centrifugal force is determined by treating 5 g of flour in a test tube with water to which

lactic acid is later added. After centrifuging and pouring off the supernatant liquid, water retention capacity is determined from the increase in weight.

Water retention capacity of Buhler experimentally milled flours is a reliable index of their loaf volume potentialities. Partial and multiple correlations indicate that water retention is an expression of both the quantity and quality of protein. The water retention values range from 150% for the flour producing the lowest loaf volume of 650 cc to 300% for the one giving the highest volume of 1365 cc.

A milling procedure involving one break and one reduction on the Hobart grinder and requiring only 11 to 12 minutes is described for 75 g wheat samples.

Water retention capacity of Hobart-milled flours is essentially a linear function of protein content within a variety. However, certain varieties have distinctly different regression lines, the slopes of which, in general, increase as water retention capacity increases.

Loaf volume of Buhler- and water retention capacity of Hobart-milled flours are very highly correlated when protein content is held constant. Accordingly, the differences between the water retention capacity regression lines apparently represent differences in protein quality.

These water retention capacity–protein content regression lines of known varieties should be useful for estimating the protein quality or breadmaking capacity of new wheat varieties.

Literature Cited

Finney, K. F.
1943 Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. 20: 381–396.
1945 Methods of estimating and the effect of variety and protein level on the baking absorption of flour. Cereal Chem. 22: 149–158.
——, and Barmore, M. A.
1945 Varietal responses to certain baking ingredients essential in evaluating the protein quality of hard winter wheats. Cereal Chem. 22: 225–243.
1945a Optimum *vs.* fixed mixing time at various potassium bromate levels in experimental bread baking. Cereal Chem. 22: 244–254.
McCluggage, M. E., Anderson, J. E., and Larmour, R. K.
1939 A comparison of the Allis-Chalmers and the Buhler automatic experimental mills. Cereal Chem. 16: 610–619.
Sharp, P. F., and Gortner, R. A.
1923 Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. Minn. Agr. Expt. Sta. Tech. Bull. 19.

COMPOSITION OF HYBRID CORN TASSELS

J. M. VAN LANEN, F. W. TANNER, JR., and SHIRLEY E. PFEIFFER

Fermentation Division, Northern Regional Research Laboratory,[1]
Peoria, Illinois

(Received for publication March 29 1946)

In the production of hybrid seed corn it is customary to plant alternately two rows to serve as the male parent and six rows to serve as the female parent plants. Tassels are removed from all female functioning plants as soon as they emerge or before pollen begins to shed, and thus before the subsequent fertilization is effected by the male functioning plants. Detasseling is carried out manually, the stalk generally being broken at the first node, and the tassels are discarded. Although the tassel comprises a relatively small portion of the total plant weight, the acreage devoted to seed production, and thus the quantity of tassels produced annually, is appreciable. Tassels of the particular strain investigated had an average dry weight of 0.033 pound per plant and since there are some 11,000 plants per acre, 75% of which are detasseled, the yield of dry tassels per acre was about 270 pounds. In 1944 there was an estimated 360,000 acres planted in hybrid seed corn which would have supplied nearly 50,000 tons of dry tassels. The tassels thus harvested can be retained at little extra cost and dried if necessary in seed corn driers which are idle during the detasseling period. It appeared desirable, therefore, to investigate their composition with a view toward utilization as a feed adjunct. Analyses were carried out during three stages of tassel development, the intermediate stage being the normal detasseling time. Also analyzed for comparative purposes were corn pollen and commercial hybrid corn grain. Tassels were found to be a good source of protein and vitamins and their possible utilization as a feed component is discussed.

Experimental Methods

About 20 tassels were taken for each analysis. Rachises below the lowest spikelet were removed so as to provide a more uniform material for analysis. It should be noted, therefore, that the analytical data presented later refer only to the tassel, rather than to the tassel, lower portion of rachis, and leaf, which are generally removed in detasseling.

Nitrogen, crude fat, and crude fiber were determined by the *Methods of Analysis* (Association of Official Agricultural Chemists, 1940). Ash was determined by holding at 550°C for four hours and moisture by drying overnight at 105°C. Carotene was measured by the method of Moore (1940) and thiamine by the fermentation method of Schultz, Atkin, and Frey (1942). The method of Snell and Strong (1939) was employed for the riboflavin assay after the samples were extracted by autoclaving at a pressure of 15 pounds for 30 minutes in the presence of 0.1 *N* hydrochloric acid. Pantothenic acid was determined by the method of Pennington, Snell, and Williams (1940) after digestion of the samples with clarase and papain for 24 hours at 37°C. Results were corrected for the vitamin contained in the enzymes. For niacin, the procedure of Snell and Wright (1941) was employed. Samples were previously hydrolyzed by autoclaving for 30 minutes at 15 pounds pressure in 0.1 *N* sodium hydroxide. For the pyridoxine assays, samples were hydrolyzed by autoclaving with 5.0 *N* sulfuric acid after which the method of Atkin, Schultz, Williams, and Frey (1943) was applied.

---

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Results

The results of proximate analyses of tassels during three stages in development are presented in Table I. Also included for comparison in Table I is the analysis of a composite sample of corn grain made up of over 100 commercial and experimental hybrids.

TABLE I

INFLUENCE OF MATURATION UPON THE COMPOSITION OF COMMERCIAL HYBRID CORN TASSELS

(DeKalb Strain 404A)

Stage of tassel development	Proximate analysis					
	Moisture [1]	Protein (*N* x 6.25)	Fat	Ash	Crude fiber	Nitrogen-free extract
	%	%	%	%	%	%
Early [2]	70.9	18.5	5.8	4.5	22.8	48.4
Intermediate [3]	55.9	18.3	6.9	4.5	18.2	52.1
Late [4]	54.6	17.1	4.3	4.6	18.5	55.5
Yellow corn, whole grain [5]	10.5	10.4	5.0	1.4	2.1	81.1

[1] Moisture is given on the "as received" basis; all other constituents on a dry basis.
[2] Tassels were entirely green and pollen was immature.
[3] Pollen mature but firmly held (normal detasseling stage).
[4] Pollen mature and released by handling tassel.
[5] Composite sample of hybrids.

The solids content of tassels increased substantially between the early and intermediate (detasseling stage) and thereafter remained relatively constant. While the highest solids content of tassels as harvested at the intermediate stage was only 44.1%, it was found that samples could be dried to more than 90% solids by exposure in the field. Except for their lower content of carotene, as will be shown later, sun-dried samples were similar to oven-dried samples in all constituents. The protein content decreased slightly during maturation but in all instances was notably high compared with the grain or other structural parts of the corn plant. The fat level rose to 6.9% at the detasseling stage, then decreased rather sharply during further development of the tassel. The ash content remained practically at the same level throughout. Early tassels were higher in crude fiber than intermediate or late tassels, probably because there was a higher proportion of rachis tissue in the immature tassels. The nitrogen-free extract increased slightly during the period of development, and at the detasseling stage it constituted approximately one-half the total dry substance.

The vitamin content of tassels (Table II) also was found to be unusually high when compared with other structural parts of the plant and, with the exception of pyridoxine, was considerably greater than that of corn grain. For example, tassels at the intermediate stage of maturation contained approximately eight times the riboflavin, three times the niacin, three times the pantothenic acid, twelve times the carotene, and twice the thiamine potency of the sample of corn grain. The pyridoxine level, however, was only about one-half that of the grain. While riboflavin and thiamine appeared to increase significantly during tassel development, the values for niacin, pantothenic acid, and pyridoxine remained relatively constant. The vitamin content of tassels which had been oven-dried was compared with those dried to 8% moisture content by exposure to the sun. Whereas no differences were noted in the B-vitamin content, the carotene level of sun-dried tassels was only 4 μg per gram or less than one-third that contained in the oven-dried material.

TABLE II

INFLUENCE OF MATURATION UPON THE VITAMIN CONTENT OF COMMERCIAL HYBRID CORN TASSELS
(DeKalb Strain 404A)

Stage of tassel development [1]	Vitamin content [2]					
	Riboflavin	Niacin	Pantothenic acid	Pyridoxine	Thiamine	Carotene
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
Early	6.5	58.0	23.0	3.9	6.1	—
Intermediate	8.8	60.0	25.9	3.2	10.0	13.0
Late	9.4	62.5	22.8	3.0	8.8	—
Yellow corn, whole grain [3]	1.1	21.7	9.1	7.4	5.4 [4]	1.1 [5]

[1] Stages of tassel development are explained in Table I.
[2] All analyses are given on a dry basis.
[3] Composite sample of hybrids.
[4] From Schultz, Atkin, and Frey (1941).
[5] From Baumgarten, Bauernfeind, and Boruff (1944).

A question arises as to the origin and location of the vitamins in the tassel since this organ is comprised of several parts which differ in physiological function such as the rachis branches, glumes, anthers, pollen, etc. Although these fractions were not separated and analyzed, the potency of pure corn pollen in several members of the B complex was determined and found to be as follows per gram of dry substance: 14.9 μg riboflavin, 81 μg niacin, 30 μg pantothenic acid, and 4.7 μg pyridoxine. Except for pyridoxine which is lower in corn pollen, these figures are in good agreement with those reported for mixed pollen by Kitzes, Schuette, and Elvehjem (1943). The vitamin content of pollen is but little greater than that of the whole tassel and does not entirely explain the high potency of the blended parts.

While the analytical data indicate that dried tassels are superior to corn and other cereal grains which are more readily available feed constituents, they are not comparable to such feed concentrates as distillers' solubles or dried skim milk in either their protein or water-soluble vitamin content. Therefore, the value of this material probably will be less than these adjuncts but will be determinable only by actual feeding trials. The potency of dried tassels in members of the B-vitamin complex suggests that they may be suitable for supplementing poultry rations.

Summary

The composition of hybrid corn tassels at various stages of maturation has been determined. Tassels are good sources of protein and vitamins, and they attain highest nutrient value at the normal detasseling time. Dried tassels may be a suitable adjunct to poultry feeds.

Acknowledgment

Acknowledgment is gratefully made to Ben W. Mattek of the DeKalb Agricultural Association for supplying corn tassel and pollen samples; to E. H. LeMense of the Agricultural Motor Fuels Division, Northern Regional Research Laboratory, for performing analyses for fat and fiber; and to Dr. W. J. Rudy of Allied Mills, Inc., for the determinations of carotene. The authors also wish to thank Dr. E. C. Lathrop of the Agricultural Residues Division, Northern Regional Research Laboratory, for valuable suggestions during the course of the investigation.

Literature Cited

Association of Official Agricultural Chemists
1940 Official and Tentative Methods of Analysis of the Association.
Atkin, L., Schultz, A. S., Williams, W. L., and Frey, C. N.
1943 Yeast microbiological methods for determination of vitamins. Ind. Eng. Chem. (Anal. Ed.) 15: 141–144.
Baumgarten, W., Bauernfeind, J. C., and Boruff, C. S.
1944 Carotenoids in corn distillers' byproducts. Ind. Eng. Chem. 36: 344–347.
Kitzes, G., Schuette, H. A., and Elvehjem, C. A.
1943 The B vitamins in honey. J. Nutrition 26: 241–250.
Moore, L. A.
1940 Determination of carotene in plant material. Ind. Eng. Chem. (Anal. Ed.) 12: 726–729.

Pennington, D., Snell, E. E., and Williams, R. J.
1940 An assay method for pantothenic acid. J. Biol. Chem. 135: 213–222.
Schultz, A. S., Atkin, L., and Frey, C. N.
1941 A preliminary survey of the vitamin B_1 content of American cereals. Cereal Chem. 18: 106–113.
1942 Determination of vitamin B_1 by yeast fermentation method. Improvements related to use of sulfite cleavage and a new fermentometer. Ind. Eng. Chem. (Anal. Ed.) 14: 35–39.
Snell, E. E., and Strong, F. M.
1939 A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.) 11: 346–350.
——, and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. 139: 675–686.

GAS PRODUCTION AND GAS RETENTION OF DOUGH AS AFFECTED BY TYPE OF FLOUR, BAKING FORMULA, AND AMOUNT OF MIXING[1]

Emery C. Swanson[2] and C. O. Swanson

Department of Milling Industry, Kansas State College, Manhattan, Kansas

(Received for publication September 17, 1945)

Gas production and gas retention are basic to the production of leavened bread. Harden (1911) found that for a given amount of yeast the rate of fermentation is almost constant, regardless of the sugar concentration, within the limits of 0.5% to 10% sugar. Johnson and Bailey (1925) believed that the gluten content of flour directly affected the gas-retaining properties of dough but did not alter the total gas production. Heald (1932) listed factors that increased gas production as: (a) increased yeast concentration; (b) sugar and diastatic malts when a deficiency of each occurs; (c) yeast foods up to a certain amount; and (d) elevated temperatures up to 95°F (35°C). Those factors which decreased gas production were: (a) salt; (b) excessive amounts of yeast food; and (c) excessive temperature. Those factors which had no apparent effect were: (a) sugar and diastatic malt when sufficient amounts were already present; (b) high speed mixing; and (c) absorption between the limits which would be used in ordinary dough fermentation. Blish and Hughes (1932) reported that maintained gas production is predominantly dependent upon the supply of fermentable sugar. They further postulated that gas production is, for all practical purposes, independent of the gas retention factor under normal baking conditions. Munz and Bailey (1936) also refer to the effect of the addition of sugar on gas production and they agree with the findings of these other workers. Bohn and Bailey

---

[1] Contribution No. 123, Department of Milling Industry.
[2] Present address: International Milling Company, Minneapolis, Minnesota.

(1936) reported that overmixing produces marked physical changes in dough properties. The extent to which flour characteristics, baking formula, and procedure influence gas retention is not so well understood as the influence of these factors on gas production.

The purpose of the present investigation was to evaluate the influence of three factors on gas production and retention, namely: flour characteristics, formula, and mixing time.

Materials and Methods

The study was limited to a comparison of three commercially milled flours, three formulas, and three mixing times. The flours comprised a soft winter, a hard red spring, and a hard red winter, which were selected as representatives of each type from a group of some twenty available samples. Selection of the three flours was made to secure differences in protein content, diastatic activity, absorption, and mixing requirements. These data are recorded in Table I.

TABLE I

Characteristics of Wheat Flours[1]

	Flour type		
	Soft winter	Spring	Hard winter
Protein, %	7.8	13.6	13.6
Ash, %	0.42	0.49	0.45
Moisture, %	11.6	11.6	11.7
Diastatic activity, mg/10 g	130	195	347
Optimum mixing time, min.	2.0[2]	2.5	3.5
Absorption (lean formula) %	54.5	72	64

[1] Data are expressed on the "as received" basis.

[2] Two minutes was arbitrarily taken as the optimum mixing time for the soft wheat flour. Actual measurements indicated a shorter time. However, the apparatus was geared to testing hard wheat flours and it was difficult to obtain a properly mixed soft wheat dough that would be differentiated from the one-minute mixing given to "undermixed" doughs. A mixing time of less than one minute was not practical because of insufficient incorporation of the ingredients and poor handling properties.

One low-sugar and two high-sugar formulas, designated respectively as "lean," "MPB," and "rich" with the ingredients shown in Table II, were used. With both high-sugar formulas the initial rapid rate of gas production was maintained for at least eight hours. The only instances in which sugar deficiencies occurred were with the "lean" formula.

The optimum mixing times were estimated from the characteristics of mixograms and by the handling properties of the dough as judged by an experienced baker. A dough was judged to be at optimum development when it had a smooth texture and dull surface, and felt dry when handled. In addition to doughs mixed for the optimum times for each flour and formula, undermixed doughs (mixed one minute) and overmixed doughs (mixed 10 minutes) were also prepared.

TABLE II

BAKING INGREDIENTS PER 100 GRAMS FLOUR

	Lean	MPB	Rich
	g	g	g
Yeast	2.5	2.5	2.5
Sugar	—	5.0	6.0
Salt	—	1.0	1.5
Dry milk solids	—	—	6.0
Malt syrup			
120°L	—	—	0.25
200°L	—	0.30	—
Ammonium phosphate	—	0.10	—
Bromate	—	1 mg	4 mg
Water		As needed	

One 5-g aliquot of each dough was used to estimate gas production, and another to estimate gas retention employing the recording gasometer recently described by Working and Swanson (1946). The data thus obtained were calculated to a 170-g dough basis, this being the average weight of the dough from 100 g of flour. Gas production data for the flours are reported as: (1) rate per hour for the initial phase, that is, up to the point where the curve breaks and the rate decreases; (2) six-hour total production; and (3) total production to the point of maximum gas retention. The latter may have considerable significance since it is dependent not only on the gas-producing ability of the dough but likewise on the gas-retaining capacity. The gas retention values recorded were the maximum quantity of gas retained and the time necessary to reach this maximum.

Results

The mean data for gas production and gas retention are given in Table III. Each numerical value represents the combined mean data from 54 determinations (three flours tested in duplicate with three formulas and three different mixing times).

Gas Production. The soft wheat flour showed the lowest initial rate of gas production and a low total production, both to the point of maximum retention and to six hours. The other two flours had very similar initial rates of production, but the high diastatic winter wheat flour maintained the initial rate of production for a longer time and, thus, gave the most total gas produced during the six-hour period. The value for total production to maximum retention was greater for the spring than for the winter wheat flour owing to the longer time required to attain maximum retention.

The type of formula had a pronounced effect on the initial rate of fermentation and on the total gas produced. The initial rates for the lean and MPB formulas were essentially the same, but the rate for the

TABLE III

EFFECT OF FLOUR TYPE, BAKING FORMULA, AND MIXING TIME ON GAS PRODUCTION AND RETENTION

(mean results for 170 g dough)

Main effects	Gas production			Gas retention	
	Initial rate	Total to		Maximum	Time to maximum
		Maximum retention	Six hours		
	ml/hr	*ml*	*ml*	*ml*	*hr*
Flour					
Soft	227	692	1149	298	3.1
Spring	239	1060	1273	451	4.6
Winter	241	961	1314	371	4.0
Formula					
Lean	250	728	1002	319	3.0
MPB	251	1048	1277	427	4.2
Rich	209	939	1457	375	4.6
Mixing time					
1 minute	234	806	1226	322	3.6
Optimum	236	1019	1253	416	4.3
10 minutes	239	889	1257	384	3.8

rich formula was much lower. The gas production values to the time of maximum retention and for six hours were highest for the MPB formula and lowest for the lean formula. This was due to the influence of the lower rate of gas production with the rich formula and to the shorter time to maximum retention with the lean formula.

Variations in dough mixing time did not influence the initial rate of gas production nor, to any appreciable extent, the six-hour production values. However, the total gas production to the point of maximum retention was largest for the optimum mixing time, smallest for the one-minute, and intermediate for the 10-minute mixing time. This is due to the effect of the mixing on the time elapsing between the beginning of fermentation and the attainment of peak retention; this time was the longest with optimum mixing and the shortest with undermixing.

Gas production, then, over either the variable time required to give peak retention or during the conventional six-hour period, is dependent primarily on the formula used. The type of flour may have an effect but this can be modified by changing the formula.

Gas Retention. Of the three flours, the spring wheat sample showed the greatest gas retention, the winter wheat flour intermediate, and the soft wheat flour the lowest. This relationship, in part, results from the dependence of gas retention, as measured, on both gas production and the time required to reach the retention peak. The soft wheat flour was deficient in both respects; the spring wheat flour was not superior to the winter in early gas production rate, but the longer period of time before the retention peak was reached permitted greater gas production and greater observed retention.

The maximum gas retention was largest for the MPB formula, smallest for the lean, and intermediate for the rich. Here again, the variation may be attributed primarily to differences in gas production. The effect of the lean formula was to give a low over-all gas production during the relatively short period before maximum gas retention was achieved. High gas production for a relatively long period of time caused the observed retention peak for the MPB formula to be high. On the other hand, with the rich formula the prolonged fermentation period before peak retention was reached was not sufficient to overcome the deficiency caused by the lower rate of gas production; as a result the retention for this formula was of intermediate degree.

Best gas retention was observed with the doughs mixed for the optimum, while the undermixed doughs showed lowest retention and the overmixed gave values of intermediate magnitude. Variation in mixing time had, essentially, no effect either on the rate of gas production or on the period of maintenance of this rate. Observed differences in maximum gas retention must therefore be due to an influence on the gas-retaining properties of the dough. Thus, gas retention is shown to be dependent primarily on gas production and mixing time, and secondarily on the flour.

Variance Analyses

Results of variance analyses of the data are summarized in Table IV. The interactions were tested by the "remainder" error term.

The variance analyses confirm the observations made from the data of Table III and only the significant interrelationships of flour, formula, and mixing time will be discussed. Figure 1 illustrates these relationships for total gas production up to the time of maximum retention. The flour-formula graph (left side) shows the superior response of the spring wheat flour to the MPB and rich formulas, the low response of the winter wheat flour, and the intermediate response of the soft wheat flour. The flour-mixing time graph (center) shows the superiority of optimum mixing time for the winter and spring wheat flours. For the soft wheat flour, there was no difference in response to the one minute and optimum mixing times, while the 10-minute mixing time was clearly damaging. The spring wheat flour was most sensitive to changes in mixing time. The formula-mixing time graph (right side) shows no effect of mixing time with the lean formula, but with the rich and MPB the optimum mixing time is clearly shown to be superior to under- or overmixing.

TABLE IV

Variance Analysis of Gas Production and Retention Data

Source of variation	D.F.	Sums of squares [1]						
		Gas production			Gas retention		Duration of initial rate of production (data from lean formula only)	
		Initial rate	Total to maximum retention	Total to six hours	Maximum	Time to maximum	D.F.	
Flours	2	2.75*	1973	846	317	34.3	2	10.92**
Formulas	2	26.57**	1436*	6536*	159	38.3*	—	—
Mixing times	2	.24	621	31	123	7.4	2	.01
Interactions								
Flour × formula	4	.52	190*	566**	17*	4.3**	—	—
Flour × mixing time	4	.80	471**	23	65**	6.2**	4	.06
Formula × mixing time	4	.17	199*	22	28**	2.4*	—	—
Remainder	143	.44	27	23	6	.5	45	.06

[1] **Denotes that variances are highly significant (exceed 1% point).
*Denotes that variances are significant (exceed 5% point).
The interactions were tested for significance against the "remainder" error term. The main effects were tested for significance by the appropriate interactions.

Figures 2 and 3 illustrate the relationships for maximum gas retention and time to maximum retention, respectively. The flour-formula graph (left) of Figure 2 shows the soft wheat flour to have the lowest gas retention for all three formulas with the spring wheat flour superior to the winter. The significance of the interaction is due to the difference in *relative* sensitivities of the flours to formula changes. The flour-mixing time graph (center) shows the greatest retention with the winter and spring wheat flours at the optimum mixing time, the least at the one minute, and intermediate retention at the 10-minute mixing time. The soft wheat flour has the least retention, the spring the most, with winter intermediate. The mixing time-formula

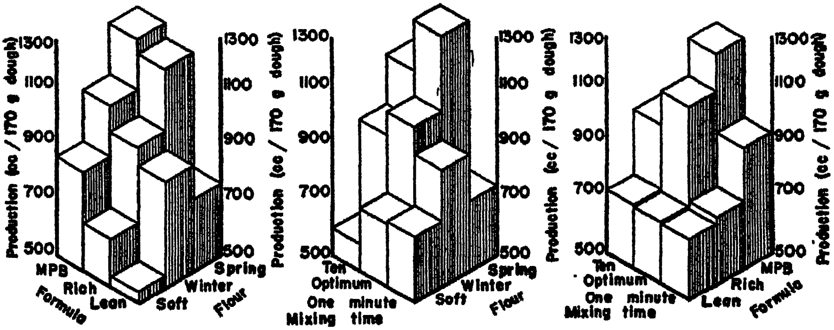


Fig. 1. Flour, formula, and mixing time relationships for total gas production up to the time of maximum retention.

graph (right) shows about the same relationship as the flour-mixing time graph (center).

The left graph of Figure 3 (time to maximum retention) shows the high sensitivity of the hard spring and soft winter wheat flours to formula changes, with the winter wheat flour least affected. The significance of the flour-mixing time interaction (center graph) was due, mainly, to the insensitivity of the flours at one-minute mixing as compared to their rather wide differentiation at either optimum or ten-minute mixing. The formula-mixing time interaction (right graph) shows the lean formula to be unaffected by variations in mixing time, while both the high-sugar formulas were markedly altered by such changes.

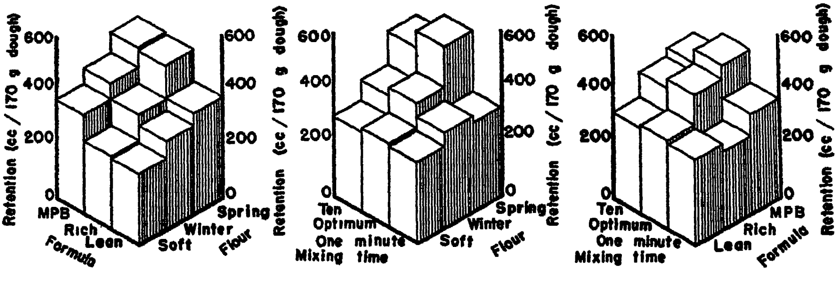


Fig. 2. Flour, formula, and mixing time relationships for maximum gas retention.

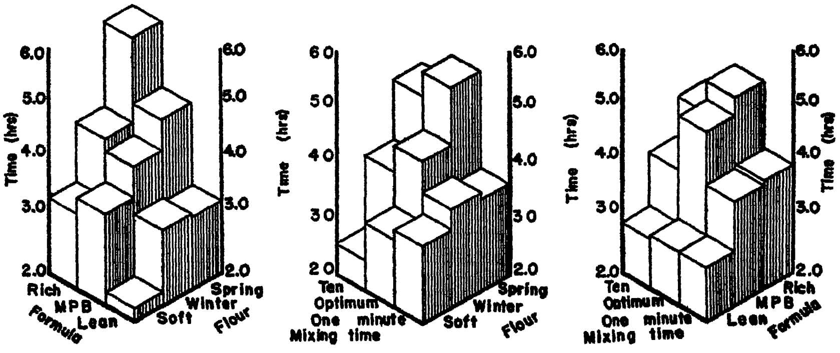


Fig 3. Flour, formula, and mixing time relationships for the time to maximum gas retention

The flour-formula interaction for the total gas production to six hours (Table IV) was highly significant. The data are given in Table V. The lean formula gave the smallest values regardless of the flours, and the MPB formula the largest, with the rich intermediate. The soft wheat flour gave the lowest values regardless of the formula, but the highest values were determined by both the flour and the formula, with the winter wheat flour having the greatest value with

TABLE V

EFFECT OF FLOUR CHARACTERISTICS AND BAKING FORMULA ON TOTAL GAS PRODUCTION

Formula	Gas produced in six hours		
	Flour		
	Soft	Winter	Spring
	ml	*ml*	*ml*
Lean	848	1220	929
MPB	1392	1460	1537
Rich	1220	1258	1370

the lean formula and the spring wheat flour the greatest with both the MPB and rich formulas.

The data show the extent to which gas retention is influenced by the rate of gas production and the duration of the initial rate. The correlation of gas production and gas retention was highest at the time of maximum retention, being + 0.83. The correlation at six hours was + 0.30. This suggests that gas production data, when used as a criterion of potential breadmaking quality, should be noted at some time less than the usual practice of six hours.

Summary

Gas production and retention data were obtained for three wheat flours varying in protein content, mixing requirement, and diastatic activity employing a "lean" (low-sugar), malt-phosphate bromate, and a rich formula and short, optimum, and long mixing times.

Gas production was dependent, primarily, on the formula used. The rich formula had the lowest rate of gas production, while the lack of fermentable sugar with the lean formula caused a marked decline in the amount of gas produced. The effect of flour type on gas production can be modified by changing the formula.

Gas retention was primarily influenced by gas production and mixing time. The higher the rate of gas production the greater the *amount* of gas retained. Optimum mixing produced a dough which had the best gas-retaining properties.

Literature Cited

Blish, M. J., and Hughes, R. C.
1932 Some effects of varying sugar concentrations in bread dough on fermentation by-products and fermentation tolerance. Cereal Chem. **9**: 331–356.
Bohn, L. J., and Bailey, C. H.
1936 Effect of mixing on the physical properties of dough. Cereal Chem. **13**: 560–575.
Harden, A.
1911 Monograph on biochemistry, alcoholic fermentation. Longmans, Green and Co., p. 107.

Heald, W. L.
1932 Some factors which affect gas production during dough fermentation. Cereal Chem. 9: 603–616.
Johnson, A. H., and Bailey, C. H.
1925 Gluten of flour and gas retention of wheat flour doughs. Cereal Chem. 2: 95–106.
Munz, E., and Bailey, C. H.
1936 Relation of amylase activity to gassing rate. Cereal Chem. 13: 427–436.
Working, E. B., and Swanson, E. C.
1946 An automatic device for the measurement of gas production and gas retention in doughs. Cereal Chem. 23: 210–216.

REPORT OF THE 1945-46 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

Frank R. Schwain, *Chairman*

Kroger Company, Columbus, Ohio

(Presented at the Annual Meeting, May 1946; received for publication May 23, 1946)

Several years have been spent in an attempt to reconcile laboratory and shop performances in cooky baking. Some correlation has been evident with our present tentative cooky formula, particularly with wire-cut goods (Schwain, 1944), but this formula has revealed a sensitivity to flour particle size not always demonstrated in the bakery. Last year, limited studies with a typical shop formula reduced to laboratory size showed better correlation with actual practice in this regard (Schwain, 1945).

Any attempt to explore the question of flour particle size this year had to be abandoned. Actual mill runs by Wilbur Hanson, one of the committeemen, demonstrated that not over 10 to 15% variation in flour particle size could be obtained from a given lot of this year's crop of Michigan white wheat.

The committee then turned to a problem which has confronted the industry for some time, namely, the tolerance range of cooky flour strength. In other words, how far apart in analyses can cooky flours be and yet produce cookies of equivalent spreading properties? It was decided to select the test flours on the basis of gluten quality or strength as defined by apparent viscosity, and to determine the actual tolerance range by cooky tests in both the laboratory and shop. A significant change in spreading properties of the cookies from any given flour was to be considered as an indication that its strength or weakness, as the case might be, was of sufficient magnitude to place it outside the range. Conversely if no appreciable change in baking response was noted, one could assume that the strength of the flour in question fell within the range of tolerance. Wire-cut types of cookies were chosen as the medium for the shops to explore in view of the previous good correlation between laboratory and bakery results with this type of piece.

Materials and Methods

Four unbleached straight-grade Michigan white wheat flours were selected which had apparent viscosities by the no-time method ranging from 22° to 34° MacMichael.

The analyses of the test flours are shown in Table I and a Ro-Tap granulation study in Table II. The "no-time" viscosity test ranks flour *A* the weakest and *D* the strongest, with *B* and *C* about equal. The "one-hour" viscosity, on the other hand, ranks *A* and *B* about on a par, with *C* stronger than these two but *D* again showing the greatest strength. It is possible that the higher mineral content of flour *A* is reflected in a lowering of its no-time viscosity. In any event a considerable range in apparent viscosity or gluten strength is indicated.

TABLE I

ANALYSES OF TEST FLOURS

Flours	Ash [1]	Protein [1]	Gluten		Maltose value [2]	Apparent viscosity	
			Wet	Dry		No-time	1 Hour
						°MacM.	°MacM.
	%	%	%	%	*mm Hg.*		
A	0.42	7.3	23.6	7.9	275	22	38
B	0.38	7.0	23.2	7.8	305	25	37
C	0.36	6.0	21.3	6.7	340	25	44
D	0.36	7.1	22.9	8.2	340	34	51

[1] 14% moisture basis.

[2] As determined by pressuremeter in 6 hour period.

TABLE II

GRANULATION OF TEST FLOURS [1]

	Percent of total flour			
	Flours			
	A	*B*	*C*	*D*
Over 11 XX Silk	0.3	0.5	3.3	0.4
Over 12 XX Silk	0.6	0.8	3.1	1.1
Over 13 XX Silk	1.4	3.7	9.4	3.9
Over 14 XX Silk	0.4	3.4	1.5	4.8
Over 15 XX Silk	7.7	14.4	15.8	15.5
Through 15 XX Silk	89.6	77.2	67.0	74.3

[1] Flours were bolted for 30 minutes by means of a Ro Tap shaker (200 g sample).

Granulation differences doubtless reflect variations in grinding and bolting operations since three different flour mills are represented.

Mixograms made from the four flours are reproduced in Figure 1. These were all made at an absorption of 52% and at a machine tension of 7. The slower development time of flour *C* is probably the result of its coarser granulation.

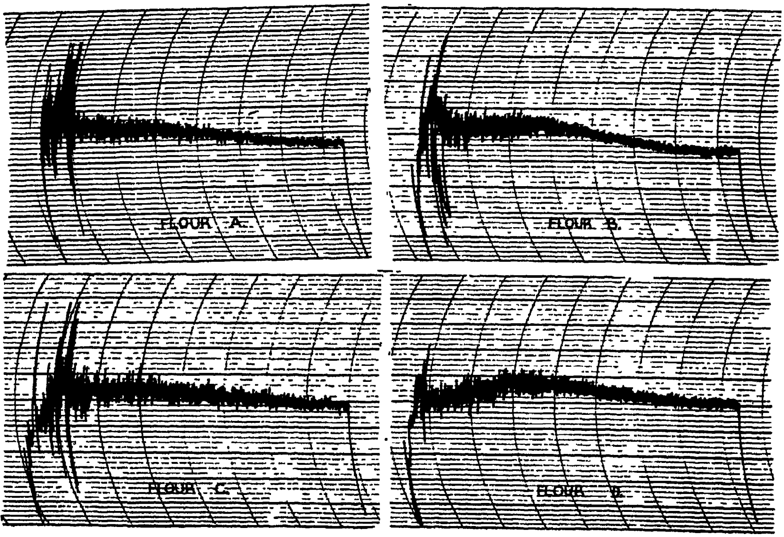


Fig. 1. Mixograms of test flours (tension 7; 52% absorption).

Cooky tests were conducted in five different laboratories and five separate shops.

The laboratory cooky baking tests were carried out in three ways. The first method consisted of the present tentative test first proposed by Hanson (1943). To this was added the modification employed by last year's committee to secure a more uniform dough consistency by controlling the amount of dry matter and the quantity of moisture added (Schwain, 1945).

The second method was fundamentally a shop wire-cut piece formula reduced to laboratory size. This is the same formula, essentially, that the committee had tried last year, except that dough consistency was controlled in the same way as in the case of the formula mentioned above. The primary difference between the present tentative laboratory formula and the shop revision was in the amount of leavening agents. The shop type utilized a far greater amount of ammonium bicarbonate and a lesser quantity of sodium bicarbonate. These changes encourage more spread than spring in the resulting cookies.

A third laboratory method consisted of a micro-cooky test developed by Karl Finney at Wooster. In this method, the dough from any given formula is mixed in a National nonrecording mixer with only two cookies baked from the same dough.

Since unpublished work has shown that the kind of metal used for cooky baking sheets produces a noticeable effect on the spring and spread of cookies, the committee decided to use a 20 gauge iron sheet in its laboratory tests. This type of metal is the same as that normally employed in baking pans for use in commercial reel type ovens.

The various bakeries were asked to employ their regular wire-cut formulas but to make no changes except possibly in absorption or, if necessary, in the setting of the wire-cut machine feeder rolls to secure satisfactory results.

Results

The results from the laboratories, recorded in Table III, show that the flours are remarkably close in cooky baking performance. Both formulas show reasonably good agreement. The revised shop formula, as was expected from its leavening balance, produced the greater spread. The micro-test shows better agreement with the revised shop formula averages than with the present tentative test. Individual results in general are not in as close agreement as in prior years.

TABLE III

LABORATORY SPREAD FACTORS (W/T) OF COOKIES MADE WITH THE PRESENT TENTATIVE TEST AND THE REVISED SHOP FORMULA

Collaborator	Spread factor of cookies							
	Flours							
	A		*B*		*C*		*D*	
	Tent.	Revd.	Tent.	Revd.	Tent.	Revd.	Tent.	Revd.
I	11.2	10.9	11.3	10.7	10.7	11.5	11.2	11.7
II	9.4	9.7	9.4	10.2	9.0	10.3	9.4	10.1
III	9.0	10.2	8.7	9.8	9.3	10.2	8.4	10.0
IV	8.9	9.6	9.1	9.7	10.0	9.6	10.4	9.9
V[1]	9.4	9.6	8.8	9.5	8.8	9.4	8.9	9.2
General average	9.6	10.0	9.5	10.0	9.6	10.2	9.7	10.2

[1] Micro cooky baking test technique employing same formulas.

Actual shop tests of the flours are summarized in Table IV.

Admittedly the choice of cookies by the various shops is not representative of the best types that could have been chosen; but the bakeries are still under considerable handicaps as far as basic ingredi-

TABLE IV

SPREAD FACTORS (W/T) OF WIRE-CUT COOKIES FROM COMMERCIAL TESTS

Collaborator	Type cooky	Flours			
		A	*B*	*C*	*D*
VI	Vanilla wafer	5.8	6.6	6.0	5.5
VII	Vanilla wafer	6.7	6.9	6.7	6.7
VIII	Oatmeal	7.6	7.7	7.6	7.6
IX	Oatmeal	8.0	8.5	-	8.0
X	Coconut	8.1	9.4	9.2	8.7
General average		7.2	7.8	7.4	7.3
Flour moisture		11.3	13.5	12.8	12.6

ents are concerned. Oatmeal doughs sometimes are not as sensitive to flour types as most other formulas. However, the average values for the shop tests show that with the exception of flour *B*, the samples are very similar in cooky making value.

It is noteworthy that flour *B* had the highest moisture and it is quite likely that this is a contributing factor to the high spread since all of the bakeries employed the flours on an "as received" basis. All the bakeries reported that the dough from flour *B* was on the soft side. Two percent additional moisture is equivalent to using 12 pounds less flour and 12 pounds more water per three barrel dough and this would naturally lead to a softer dough with more spread.

This suggests that it would be advantageous for bake shops to weigh flour into the mixers with a weight standardized to some predetermined moisture level, say 12%. Such a procedure might conceivably level out flour performance; it would certainly aid in the attainment of more uniform goods, and should reduce invisible losses which ultimately affect yield.

Collaborator X had sufficient flour to repeat the flour series, in which he prepared each dough by employing a constant weight of flour as computed on a 12% moisture basis. The results are found in Table V.

TABLE V

COMPARISON OF W/T FACTORS FOR WIRE-CUT COOKIES FROM COMMERCIAL DOUGHS CORRECTED AND UNCORRECTED FOR FLOUR MOISTURES

Dough preparation	Flours			
	A	*B*	*C*	*D*
Flour weighed "as received"	8.1	9.4	9.2	8.7
Flour weight corrected to 12% moisture basis	9.6[1]	9.5	9.7	9.5[1]

[1] Average of several doughs.

When moisture is taken into account in the shop, the performance of the different flours is much more uniform. This is in harmony with the laboratory predictions. Flours *A* and *D* were baked in duplicate in the shop and the replicability of the tests on flour *A* was very unsatisfactory; W/T values of 10.15 and 9.0 were secured in successive days under strict supervision. This points clearly to the fact that the shop data, which in most cases represents single doughs, may not reflect what would be found as a result of a large number of tests where such unavoidable variations as personnel, machine, and baking conditions would tend to be overcome.

Conclusions and Recommendations

From these studies it would appear that unbleached Michigan white wheat straight grade flours may vary in apparent viscosity as much as 10° MacMichael on the basis of the no-time method or approximately 15° MacMichael with the one-hour digestion test and still produce wire-cut cookies with comparable spreading properties. This has been demonstrated in laboratory tests and somewhat limited commercial runs. The latter showed quite clearly that the performance of flours may be markedly affected by differences in moisture content, particularly if the moisture variable is overlooked by the bakery in weighing the flours for the doughs.

Since moisture is usually neglected in the shop, it is well for the mill chemist to keep this variable in mind should a criticism be leveled against his flour when compared to another similar type. Conversely, the cooky bakeries would do well to consider the moisture factor when weighing the flour since it is obvious that to ignore it is to invite variations in performance, quality, and actual yield.

Both laboratory formulas agree fairly well in evaluating the flours. The results support the view held by many soft wheat workers that no single physical or chemical test will define the cooky performance of flours, and that the baking test is one of the most dependable guides.

It is recommended that future committees continue to work toward an official cooky test bake formula, studying both formulas used here, with the object of eventually recommending the one which is most suitable. The ammonium bicarbonate tolerance of a flour might be investigated. The range of cooky flour strength employing flours with differences in viscosity of about 15° MacMichael (no-time), if such a spread can be obtained from Michigan white wheat, should be further explored. Closer agreement in laboratory results was secured in prior years when only two or three flours were studied than in the present study which involved a heavier schedule. Shop test accuracy would also be improved by making replicate tests with fewer flours.

Acknowledgments

The committee acknowledges the contributions of the Hayden Milling Company, Hankel Flour Mills, and F. W. Stock and Sons, in supplying the flours. Appreciation is also due Laurel Biscuit Company, Perfection Biscuit Company, Sawyer and Strietmann Divisions of the United Biscuit Company of America, and the Columbus Unit of the Kroger Company for extending their facilities for the commercial tests. The chairman wishes to express personal appreciation to the members of his committee: Pearl Brown, Karl Finney, William Hanks, Wilbur Hanson, Paul Hodler, Thomas Hollingshead, Larry Luedemann, Jan Micka, Howard Simmons, Otho Skaer, and Lee Thomson. Special credit is due Wilbur Hanson and Paul Hodler for conducting supplementary tests.

Literature Cited

Hanson, W. H.
1943 Report of the 1941–42 committee on testing biscuit and cracker flours. Cereal Chem. 20: 314–318.
Schwain, F. R.
1944 Report of the 1943–44 committee on testing biscuit and cracker flours. Cereal Chem. 21: 407–412.
1945 Report of the 1944–45 committee on testing biscuit and cracker flours. Cereal Chem. 22: 553–558.

BOOK REVIEW

Studies on the Nature of the Bromate Effect. By Holger Jørgensen. 435 pp. English Edition published in 1945 by Einar Munksgaard, Copenhagen, Denmark, and Humprey Milford, Oxford University Press, London, England. Price "Dan. Kr. 40."

In this voluminous text Holger Jørgensen reviews and amplifies his previous work on the mechanism of the action of bromate and other improvers on flour. The English edition published in 1945 is a translation from the Danish by Einar Christensen of Jørgensen's doctoral thesis published in Copenhagen in 1941.

The book consists of six introductory chapters, three main sections, an appendix describing the flour inventory, and finally a bibliography. The first main section is concerned with the methods used in the investigation. Baking test procedures and methods for the determination of nitrogen, moisture, ash, diastatic activity, hydrogen ion concentration, and oxidation-reduction potential are discussed in minute detail. Techniques employed for the measurement of the activity of papain and similar proteolytic enzymes by means of both gelation and extraction tests are fully outlined. In the second section the author cites numerous experiments supporting his contention that the beneficial effect of bromate and other improvers is due to inhibition of the flour proteinases which have been activated by the presence of yeast. The arguments he gives in support of this theory are as follows:

"(1) It can be established indisputably by tests that the action of the oxidizing agents which improve the baking strength is *not* due to the circumstance that these substances stimulate the CO_2 production of the yeast. Thus we may reject as erroneous one of the oldest theories for the bromate effect, viz., that bromate incites the yeast to a stronger CO_2 production.

(2) It can be established by test that $KBrO_3$, KIO_3, $(NH_4)_2S_2O_8$ and $NaBO_3, 4H_2O$ (thus all of the oxidizing improvers) strongly inhibit the gelatin-splitting ability of papain and its related enzyme, bromelin. $KClO_3$, which does not improve the baking strength, does not inhibit the action of these proteinases.

(3) When extracting flour with water (autolysis) the improvers $KBrO_3$ and KIO_3 cause a diminished solubility of the N of the flour, while the not-improving oxidizing agent $KClO_3$ does not have this effect on the N-solubility.

(4) The depression of the N-solubility due to the addition of $KBrO_3$ and KIO_3 mentioned under (3) increases very considerably if the extraction is carried out on wheat flour which has been made strongly proteolytic by the addition of papain. $KClO_3$, however, remains passive.

(5) It can be established by test that the proteolytic influence of wheat germ and wheat malt on gelatin is inhibited by $KBrO_3$ and KIO_3, but *not* by $KClO_3$.
(6) Ascorbic acid—a substance of which it is known that it can inhibit proteinases of the papain group—has been found to act improving on the baking strength.
(7) Glutathione—a substance of which it is known that it activates proteinases of the papain group—has been found to destroy the baking strength.
(8) By its presence in dough, yeast causes an activation of the proteinases of the flour."

The third main section is a critical review of 23 papers by other investigators dealing with Jorgensen's theory.

It has been demonstrated by numerous investigators that proteolytic enzymes are present in wheat and, as might be expected, they occur in a smaller amount in patent flour than in other milled fractions of the wheat. Moreover, all evidence points strongly to the fact that the proteolytic enzymes of wheat belong to the papain group. Previous workers have shown that enzymes classified in the papain group are activated by sulfhydryl compounds and cyanide and inactivated by certain oxidizing agents or other compounds containing elements such as copper or mercury that can combine with the sulfhydryl group of the enzyme. But the fact that certain oxidizing and reducing agents act solely by inhibiting or activating the proteolytic enzymes of flour is difficult to prove, because every method of measuring the change in the proteolytic activity of flour, as applied to the complex conditions existing in breadmaking, is open to some criticism. The proteolytic activity of patent flour is small and differences with various flour treatments are not significant unless levels many times those used in commercial practice are employed. Jørgensen has attempted to answer this criticism by pointing out that the ratio of water to flour is much greater in his extraction experiments than in dough and that the presence of yeast in dough would further activate the enzymes and hence less oxidizing agent will be required. However, it appears to the reviewer that both these arguments prove the opposite. In solution there should be a greater chance for a more complete reaction between the enzyme and the oxidizing agent than in a dough. Since yeast activates the proteolytic enzymes of flour, one would think that greater amounts of oxidizing agents would be required in dough than in the extraction tests; yet the opposite is the case. Secondly, gluten is a most difficult substrate to use for the measurement of proteolytic activity. Most workers in this field have had to resort to the use of foreign substrates such as casein or gelatin. Jørgensen has leaned heavily on two techniques for demonstrating his thesis. One is an extraction procedure in which flour is autodigested in aqueous suspension with and without the improver and the difference in the amount of soluble nitrogen in aliquots of the centrifuged, filtered extracts is determined. The other method is a procedure whereby proteolytic activity is measured qualitatively by the ability of the flour suspension, with or without additions, to change the time of solidification of gelatin.

Jørgensen's weakest arguments are those listed under (2), (4), and (7). The inhibition of the gelatin-splitting ability of papain and bromelin by bromate, iodate, ammonium persulfate, and sodium perborate is certainly only circumstantial evidence as applied to flour. Furthermore, the fact that the depression of the nitrogen solubility, due to the addition of bromate, increases very considerably if the extraction is carried out on wheat flour made strongly proteolytic by the addition of papain does not provide an explanation of the extent of the effect of the proteolytic enzymes of the flour itself. In regard to the argument (7), it is quite true the glutathione activates the proteolytic enzymes. However, it is still an open question whether or not a direct chemical effect of sulfhydryl compounds might be more responsible. In addition, although it can be demonstrated that a reducing medium is present in dough, we have never been able to secure a positive nitroprusside test on a fermenting dough.

In the third main section of this book Jørgensen critically evaluates the work of other investigators in this field and frequently uses their data to support his own beliefs, although this may not always be justified. One of the papers most kindly reviewed was from this laboratory and there are a few points in regard to Jørgensen's criticisms of this paper that should be mentioned. We stated that the detrimental effect of wheat germ on the baking quality of flour was due largely to its content of glutathione. But Jørgensen says, after describing an experiment wherein wheat germ but not glutathione liquefied gelatin, "Thus it is absolutely proved that wheat germ, besides glutathione, contains a proteinase, and it would be wrong to assume that the germ's proteinases do not participate at all in wheat germ's destruction of the baking strength Considering the large glutathione content which Sullivan and co-workers found in the wheat germ, it is believed, though, that a large part of the detrimental effect of wheat germ must be attributed to the glutathione content." There is no argument whatever that wheat germ contains proteolytic enzymes; it is a well-known fact. The point brought out in our paper was that a water extract of germ (containing both glutathione and proteolytic enzymes), which was boiled for 10 to 15 minutes, still had a bad effect and boiling surely inactivated the proteolytic activity. This behavior can be demonstrated by farinograph curves and actual baking tests, as was shown in an article published in *Cereal Chemistry*, **14**: 489–495 (1937). Again, in the original article which Jorgensen reviewed, we stated, "It is interesting to observe in this connection that cysteine, which contains the specific -SH group, causes a very marked deleterious action as measured by the farinograph However, unlike glutathione, cysteine permits the production of a normal loaf of bread." Jorgensen then proceeds to show that cysteine, when used at the rate of 100 mg per 560 g of flour, produced a loaf 20% smaller than the loaf without cysteine. In all our experiments cysteine caused a pronounced stickiness in the dough, as is also indicated in the farinograph curve, but when employed at the same level of -SH as glutathione, the loaves containing cysteine were practically normal in volume (only 3 to 5% lower than the standard); whereas the loaves made from flour to which glutathione was added were 20% or more below the untreated flour. Even when both compounds were added at the same level on a weight basis, provided too high an amount was not employed, the glutathione invariably gave the poorer loaf. Obviously, however, if a high enough level of cysteine is used, a poor volume will result.

Jørgensen reviewed only selected articles published to 1940, so it would be unfair to discuss in this review work that has appeared during the last five years. I wonder, however, what comments he would make on the papers published by Olcott, Sapirstein, and Blish, *Cereal Chemistry*, 20: 87–97 (1943) and by Sandstedt and Fortmann, *Cereal Chemistry*, 20: 517–528 (1943).

There are needless repetitions in the book and quite a little irrelevant material such as measurements of the oxidation-reduction potential of spinach juice. But I was much interested in the use of bromate in preventing the blowing of cheese.

The mechanism of the action of bromate and other improvers is a complex and intriguing problem. Whatever one may think of Jørgensen's firm conviction that the effect of certain oxidizing and reducing agents on flour is satisfactorily explained by his theory, it must be admitted that his work has contributed greatly to the solution of the problem and stimulated much needed research in this field.

BETTY SULLIVAN
Russell Miller Milling Company
Minneapolis, Minnesota

CEREAL CHEMISTRY

VOL. XXIII SEPTEMBER, 1946 No. 5

GRAIN STORAGE STUDIES. IV. BIOLOGICAL AND CHEMICAL FACTORS INVOLVED IN THE SPONTANEOUS HEATING OF SOYBEANS[1]

MAX MILNER[2] and W. F. GEDDES[3]

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minnesota

(Received for publication May 21, 1946)

Observations on the spontaneous heating of grain have usually been made with seeds stored in bulk. Until the work of Ramstad and Geddes (1942) and of Sallans, Sinclair, and Larmour (1944) who carried out heating studies under virtually adiabatic conditions, the few laboratory studies which have been reported have utilized either poorly insulated lots of grain or Dewar flasks.

The fundamental causes of the spontaneous heating of grain have not been clearly elucidated. The conclusion of Bailey and Gurjar (1918) and Bailey (1940) that the heating of stored wheat and other grains is due to seed embryo respiration has been questioned by several investigators who have pointed out that molds resident on normal grain may proliferate at storage moisture levels, and that their metabolic and respiratory activities are strongly thermogenic. Cohn (1890) early found that germinating seeds maintained in an insulated box would reach temperatures of only 40°C due to the thermogenic effects of seed respiration under germinating conditions. At this temperature the seedlings were killed. He attributed subsequent temperature increases to 65°C to thermophilic microorganisms. Darsie, Elliot, and Peirce (1914) carried out similar studies in Dewar flasks and pointed out that temperature increases due to the respiration of germinating seeds were slight compared to those caused by mold growth. Gilman and Barron (1930) concluded that "in bins of stored grain, marked increases in temperature may be ascribed to mold growth."

---

[1] Paper No. 2304, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, March, 1945.

[2] Research Associate, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

[3] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

Recent literature on the thermogenic activity of microflora growing on a variety of moist agricultural materials has not been adequately related to spontaneous heating phenomena in stored grain. Among such studies are those of James (1927), James, Rettger, and Thom (1928), Bakke and Henson (1933), Bakke and Noecker (1933), Harrison (1934), Carlyle (1940), Wedberg and Rettger (1941), Norman, Richards, and Carlyle (1941), and Carlyle and Norman (1941). Browne (1929, 1933), Haldane and Makgill (1934), and Hoffman (1935, 1940) have speculated on the relationship of microfloral heating to spontaneous combustion in biological materials. Most of the studies cited were carried out at high moisture values (usually about 30%) where numerous types of molds, yeasts, and bacteria proliferated. They have indicated that two ranges of temperature exist due to microfloral heating. The first range, attributed to the metabolic and respiratory activity of mesophilic nonspore formers, in which class the molds are included, ends at about 50°–55°C, while a secondary heating to a maximum of 70°C is due to thermophilic bacteria. Milner and Geddes (1946) have emphasized that only certain mold species, namely those which will proliferate at relatively low humidities, are important in the respiratory activity of stored grain. Several writers have indicated that bacterial growth is not to be expected on natural products at humidity levels below 95%.

With a view to obtaining more precise information on the nature and course of the spontaneous heating of soybeans than was available, the adiabatically controlled apparatus of Ramstad and Geddes (1942) was refined and its utility extended by the addition of apparatus for the control of aeration of the heating samples, in such a manner that the respiratory characteristics could be precisely followed. In addition to studies on the spontaneous heating of normal soybeans at moisture levels favoring storage deterioration, adiabatic trials were carried out with pure cultures of microflora isolated from soybeans and inoculated on sterile seeds.

Apparatus

Two adiabatically controlled respirometer units, schematically represented in Figure 1, were used. The insulated air bath *A* consisted of a wooden thermostat covered with two layers of celotex. Dewar flask *D*, which may be one quart, one pint, or half-pint in size, contains the seeds whose heating characteristics are to be studied. Copper-constantan thermopile *T* has 24 junctions, 12 in the seed sample and 12 exposed on the outside of the Dewar flask to the air of the bath. Slight heating of the seeds in *D*, causing a temperature differential to exist between the two ends of the thermopile, results in a flow of electric current through the coil of a sensitive D'Arsonval galvanometer *G* to which a mirror is attached, which in turn rotates a reflected light beam from a fixed integral light source along path *X* to a photoelectric cell actuating a relay in box *R*, which controls heater coils (*H*) in the air bath. The air in the thermostat is circulated continuously over the heaters by fan *S* and motor *M* through holes in the baffle *B*. When the air temperature of the bath reaches that of the seeds in the Dewar flask, the current in the thermopile circuit falls to zero and the torsion of the galvanometer coil suspension causes the light beam to recede from the photocell, thus interrupting the current to the heaters. The temperature of the air bath is thus continuously in step with that of the seeds in the Dewar flask, which are thus allowed to heat under virtually adiabatic conditions. A continuous curve of seed heating may be obtained over periods of days or weeks by simply reading the thermometer *V* inserted in the air bath at regular intervals. Not shown in the diagram is an auxiliary heater controlled by an external rheostat, which is used to compensate for heat

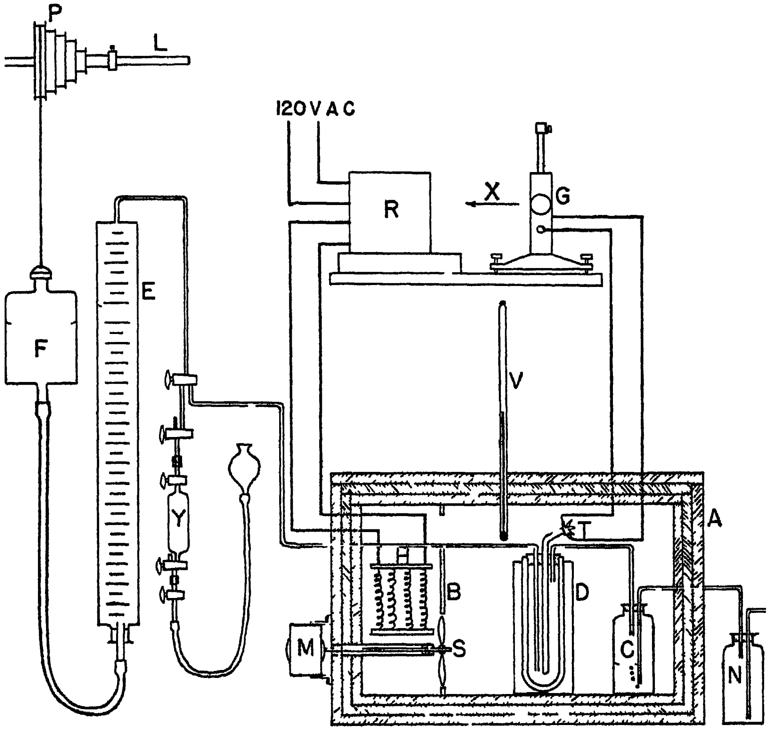


Fig. 1. Diagram of apparatus used to study the spontaneous heating and respiration of seeds.

losses from the system as the temperature of the apparatus rises. In practice, the apparatus is initially calibrated by placing warm water (45°–60°C) in the Dewar flask and adjusting the galvanometer suspension torsion until the bath actually cools about 0.2°C in 24 hours. Conditions are thus not strictly adiabatic, but any possibility of the seeds being heated by the system in this temperature range is thereby avoided. At the end of a heating trial with seeds, the apparatus is again checked with warm water in the same manner.

Aeration of the seeds in the Dewar flask is accomplished by the same system as described by Milner and Geddes (1945) in their studies on soybean respiration at constant temperature. Leveling bulb *F* moving downward slowly due to the slow rotation of pulley *P* and lineshaft *L* (controlled by a reduction gear motor and chain drive not shown in the diagram) causes the calcium chloride solution in the graduated spirometer *E* to fall, thus drawing outside air at a continuous, constant rate through the soda lime bottle *N*, the humidifying solution in *C*, and through the seeds in Dewar flask *D*. Pulley *P* has steps of various diameter, making possible daily aeration rates of from 250 to 2,000 ml. Entering air streams were humidified by passage through sulfuric acid solutions of the appropriate vapor pressure to yield a relative humidity of the air which was in equilibrium with the seed moisture value (Milner and Geddes, 1945). Samples of the daily accumulation of respired air in *E* are withdrawn for analysis into gas sample tube *Y* by means of the stopcocks and mercury leveling bulb shown. The air samples were analyzed daily for oxygen and carbon dioxide using the Haldane-Henderson gas analyzer following the method outlined by Milner and Geddes (1945).

To obtain information on chemical and microfloral changes in the heating seeds as a heating trial progressed, a number of small bottles (not shown in the sketch), containing subsamples of the seed used in the Dewar flask, were placed in the air bath and aerated by means of an independent siphon bottle system. By this procedure, control of the temperature in the air bath was accomplished by the seeds heating in the Dewar flask, while small samples of the same grain, subjected to the same temperature changes, could be removed periodically for chemical and microfloral examination.

Anomalous Behavior of Thermopile-Galvanometer Controlled Adiabatic Thermostats. During the course of the present studies, it was noticed that calibration of the adiabatic thermostats with warm water to maintain either constant temperature or a slight cooling rate, at any given initial temperature, did not yield the same cooling rate when water of a different temperature was used. Thus, for example, if the instrument were adjusted at 25°C to cool approximately 0.1°C per 24 hours, the same calibration at 50°C would yield a cooling rate of about 0.5°C per 24 hours, in spite of the fact that at both temperatures the fixed heaters in the thermostat maintained the temperature of the air to within a few degrees of that of the water in the Dewar bottle. Conversely, a small cooling rate adjustment at 50°C (0.1° to 0.2°C per 24 hours) would result in slight *heating* of the system at 25°C in the order of 0.2° to 0.4°C in 24 hours. Attempts to overcome this difficulty were unsuccessful.

Among the factors investigated which might have a bearing on this phenomenon were: (1) thermal-electric effect in thermopiles, which is manifested by a nonlinear response of the thermopile current to temperature; (2) heat conduction along thermopile leads; (3) change of resistance and current of thermopile circuit with temperature; and (4) radiant heating effects in the system. None of these factors appeared to be involved in this anomalous behavior.

This phenomenon did not nullify the value of the adiabatic technique, inasmuch as its effects were recognized. Generally, the adiabatic thermostats were calibrated at 45° to 60°C to cool about 0.2° to 0.5°C in 24 hours. Small heating effects due to calibration were therefore likely to occur with seeds at the beginning of a heating trial at room temperature, but these were manifested by linear or slightly hyperbolic temperature increases. In contrast, temperature-time curves yielded by spontaneous heating phenomena were always *exponential* in form. Accordingly, heating effects obtained from room temperature to that at which the thermostat was calibrated (45° to 60°C) would be a composite of a slight and virtually linear heating effect due to the calibration anomaly, and a large and exponential form of heating due to biological agencies. Furthermore, heating effects in temperature ranges at and above the calibration temperature (45° to 60°C) are not only entirely due to spontaneous thermogenic processes in the seeds, but are at the same time underestimations of the true maximum heating potential, inasmuch as the heat loss from the system increases as the temperature exceeds the calibration temperature.

Materials and Methods

Materials. The soybeans used in this study included: (1) a sound sample of Illini soybeans of the 1942 crop, testing 94% germination, grown in Illinois, and (2) an exceptionally sound, well-matured sample of Wisconsin Manchu soybeans grown for seed purposes at University Farm, St. Paul, Minnesota, in 1943, and testing 93 to 95% germination.

METHODS:

Conditioning Soybean Samples. The seed samples were conditioned to the required moisture levels by the direct addition of water;

moisture values were determined by means of a two-stage method as described in a previous communication (Milner and Geddes, 1945).

Examination of Soybeans for Fungi. Estimation of the number of seeds in a given sample infected with molds, as well as identification of the prevalent mold types, was carried out by the surface-sterilization and plating technique described by Milner, Warshowsky, Tervet, and Geddes (1943).

Chemical Analyses. Analyses for crude protein, crude ether extract, and for total, reducing, and nonreducing sugars were carried out on finely ground soybean samples by the methods outlined for wheat flour in *Cereal Laboratory Methods* (4th ed., 1941). Nonprotein nitrogen was measured by the trichloracetic acid method described by Becker, Milner, and Nagel (1940).

Spontaneous Heating and Respiration of Illini Soybeans

A study of the spontaneous heating and the respiratory phenomena exhibited by Illini soybeans was carried out with a sample weight of 550 g of seed at an initial moisture value of 22.8% (equilibrium relative humidity approximately 92%). A number of 100-g portions of the same bulk sample in small bottles were placed in the air bath and independently aerated for the purpose of sampling the seeds at various stages in the heating trial.

The data for this trial are given graphically in Figure 2. In this and subsequent figures, the scale for daily rates of carbon dioxide production is shown on the left margin while that for the daily recorded temperature is shown on the right. An additional graph for the respiratory quotients is shown at the top of each figure. Chemical and germination values, and the degree of microfloral infection, at various intervals in the trial, are shown in Table I.

The initial aeration rate of 2,000 ml per day proved to be insufficient, as respiratory inhibition was apparent from the slope of the respiration curve during the first few days. On the seventh day when the interseed air carbon dioxide and oxygen values had reached 18.8% and 2.2%, respectively, the aeration rate was doubled by increasing the speed of the aeration device, and samples of the respired air were accordingly taken twice daily until the end of the experiment.

The moisture content of the seeds in the Dewar flask at the end of the trial was 22.1% (initial value 22.8%). The initial and final weights of the sample at these moisture values were 550 and 542.5 g, respectively, representing dry matter weights of 428.4 and 424.6 g. The apparent dry matter loss during the trial was therefore 3.8 g.

A calibration check of the adiabatic thermostat at the end of the trial showed it to be cooling at the rate of 0.3°C per 24 hours from an initial water temperature of 55.8°C.

The data show clearly that the parallel increases in temperature and carbon dioxide production are associated with an increasing mold population until a temperature of 50°C is reached. At this point, thermal killing of the biological respiratory agencies sets in with a corresponding rapid decrease in respiration. During this initial heat-

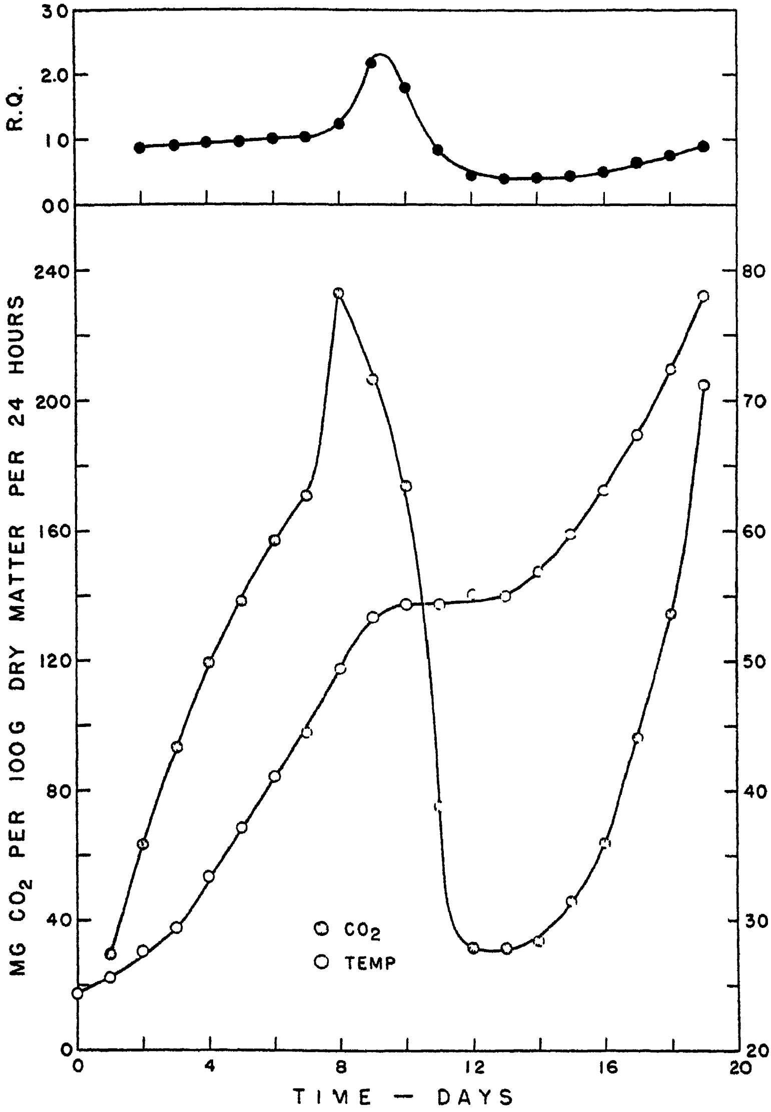


Fig. 2. The course of spontaneous adiabatic heating, respiration, and respiratory quotient exhibited by Illini soybeans, under adiabatic conditions, at 22.8% moisture. The aeration was doubled on the seventh day.

ing phase the respiratory quotient approaches a value of 1.0 which in this case was attained immediately prior to thermal killing. This regular increase in the respiratory quotient is probably indicative of the increasing contribution of the molds to the total respiration, as pointed out in previous studies at constant temperatures by Milner and Geddes (1945). As the temperature increased beyond this point, the respiratory quotient rose sharply to a value in excess of 2.0, and then decreased as rapidly, this decrease coinciding with the thermal killing of the seeds and molds. At this point, the strong sour odor, characteristic of heating grain, first became apparent in the respired gases. The inhibition of the biological respiratory agencies caused the heating curve to reach a plateau; after a few days, however, a secondary phase of spontaneous heating accompanied by an increase in carbon dioxide production appeared. In view of the complete fungal sterility of the seeds after the 15th day (Table I) this can be ascribed only to spontaneous chemical oxidation.[4] In this stage the rapidly increasing rates of heating and carbon dioxide production gave no signs of reversal, or that their upper limits had been reached when the experi-

TABLE I

INFLUENCE OF SPONTANEOUS HEATING ON CHEMICAL COMPOSITION, MICROFLORAL INFECTION, AND GERMINATION OF ILLINI SOYBEANS

Days	Temp.	Chemical analyses							Micro-flora	Germination
		Crude protein (N × 6.25 dry basis)	Non-protein nitrogen	Sugars				Crude ether extract (dry basis)	Seeds infected with fungi[2]	
				Total[1]	Non-reducing as sucrose	Reducing as glucose	Reducing as % of total			
	°C	*%*	*mg/10 g*	*mg/10 g*	*mg/10 g*	*mg/10 g*	*%*	*%*	*%*	*%*
0	24.4	35.4	22.4	444	400	44	10.0	21.6	3	93
3	29.4	35.4	21.2	410	378	32	7.8	19.5	11	73
5	37.1	35.8	21.2	405	373	32	6.9	20.2	16	52
7	44.4	35.1	22.2	378	347	31	8.1	20.2	27	30
8	49.3	35.2	23.6	433	382	51	11.8	20.3	64	0
10	53.7	35.4	28.2	417	334	83	19.9	19.4	40	0
12	55.1	35.7	34.7	491	245	246	50.1	15.3	14	0
13	55.2	35.8	32.4	438	251	187	42.6	14.9	13	0
15	59.7	36.1	32.3	443	226	217	48.9	13.6	9	0
17	67.3	36.3	35.7	432	172	260	60.3	12.0	0	0
19	77.0	36.6	35.0	291	27	264	90.8	10.5	0	0
Dewar[3] flask	77.0	36.6	37.7	310	59	251	81.0	11.2	0	0

[1] Sum of sucrose and glucose.
[2] The majority of the fungi were *Aspergillus* spp
[3] Sample analyzed after 19 days.

---

[4] Subsequent experiments showed that the bacteria indigenous to these soybeans would not proliferate at this humidity level.

ment was terminated on the 19th day. In this last stage of spontaneous chemical heating, the respiratory quotient, which had fallen to extremely low values (0.4 to 0.5) following thermal inactivation of respiration, rose slowly as the chemical heating increased. It is noteworthy that this range of 0.4 to 0.5 in the respiratory quotient agrees precisely with the values obtained by Milner and Geddes (1945) in respiratory trials with soybeans at 50°C.

These results serve to elucidate the cause of failure of previous studies on microbiological thermogenesis and spontaneous heating to yield temperatures exceeding 55°C where molds or mesophilic bacteria are the cause of heating, or above 70°C where thermopiles are involved. The plateau reached by the heating curve due to the cessation of biological respiration indicates that rather strict adiabatic conditions must be maintained in order that the spontaneous chemical oxidation, which is very weak at these low temperatures, may be allowed to develop. Sallans, Sinclair, and Larmour (1944) carried out studies on the spontaneous heating of flaxseed and sunflower seed in an adiabatically controlled thermostat based on the design of Working as described by Ramstad and Geddes (1942). A maximum temperature of only 52°C, a value within the mold thermal death range, was attained. It would appear that adequate aeration was not provided. These workers point out that under conditions of oxygen starvation, a regular relationship between heating and respiration rate as measured by carbon dioxide production is not to be expected. However, in the present studies where aeration was usually sufficient to maintain interseed carbon dioxide concentrations below levels inhibitory to respiration (Milner and Geddes, 1945), a direct relationship between temperature and respiratory increases was observed.

Carlyle and Norman (1941) also used an adiabatically controlled thermostat in their studies on microbial thermogenesis. These workers were unable to show heating beyond 70°C in experiments involving thermophilic bacteria growing on hay at 100% relative humidity.

The present study seems to add confirmation to the data and theories of Haldane and Makgill (1934) and Hoffman (1935, 1940) concerning the phenomenon of spontaneous chemical oxidation. The former writers held moist hay at temperatures just beyond the thermal death point of microorganisms and found that the gas exchange of the chemically oxidizing material was characterized by a very low respiratory quotient. Hoffman confirmed this observation, but showed in addition that previously heated sterile hay would give increasingly higher respiratory quotients if held at successively higher temperatures. Both of these observations are capable of explanation from the data obtained in the present trial with soybeans, in which the heating beyond the microfloral death range was entirely spontaneous. At temperatures slightly above that required to kill molds (50°–55°C), the respiratory quotients were very low (0.4 to 0.5), indicative of strong oxygen absorption and low carbon dioxide evolution. Higher temperatures apparently cause a breakdown of the previously formed, highly oxidized materials to yield a strong evolution of carbon dioxide. The oxygen requirement for complete oxidation in this stage would be correspondingly low, with the result that the respiratory quotient would rise.

The germination data, chemical analyses, and degree of mold infection (Table I) obtained on samples removed at various stages during the trial lend support to the foregoing interpretation of the heating and respiratory changes. The slight increase noted in the protein content is probably a reflection of a decrease in sample dry matter weight due to selective respiratory utilization of carbohydrates. Total sugars show a slow decrease in the initial biological heating stage to 49.3°C followed by a sharp rise in the initial phase of spontaneous chemical heating. This increase may only be an apparent one since it might well be due to thermal cleavage of large carbohydrate molecules such as sucrose, stachyose, raffinose, and pentosans, to yield numerous small reducing fragments. Such reducing fragments would absorb oxygen strongly which might account in large measure for the low respiratory quotient of the gas exchange in this phase. The succeeding heating period then brings about a rapid decline in total sugars. Nonreducing substances calculated as sucrose show a slow decline in the biological stage of heating and a marked decline as chemical heating sets in. Reducing substances similarly decline slightly in the biological heating phase but increase drastically at the expense of the nonreducing carbohydrates in the region of spontaneous chemical heating. The reducing sugar values in this phase may be an index of the thermal oxidative cleavage already discussed.

The crude fat fraction showed virtually no change until the temperature-time curve reached a plateau signifying the end of biological respiration. From this point the extractable oil fell sharply from 20.3% to 10.5% at the end of the trial when the temperature had reached 77°C. If it were assumed that this loss of oil were due to respiration, a disappearance of $\frac{(20.3 - 10.5)}{100} \times 428.4$ or 42.0 g of dry matter would be expected. As indicated previously, the actual loss of dry matter was only 3.8 g. Assuming a respiratory quotient for carbohydrates of 1.0, the total carbon dioxide evolution over the course of the trial would require a loss of 6.1 g in dry weight. It must be concluded that not only was the oil fraction *not* materially diminished, but that the loss of carbohydrate was less than would be expected on the basis of carbon dioxide evolution. It seems possible that the low respiratory quotient of the gas exchange which occurred following biological inhibition may in part be associated with an absorption of oxygen by the unsaturated soybean oils, to yield oxidation polymers. Such polymers would be only slightly soluble in petroleum ether.

This experiment indicates that the initial stage of heating in damp seeds may be largely attributed to mold activity. The temperature at which biological respiration was inactivated corresponds closely to that found by Carlyle and Norman (1941) for several mold species which they designated as mesophils. The study has experimentally demonstrated that spontaneous heating occurs in biological materials, following the thermal killing of the microflora initially present. Browne (1933) has pointed out that this heating due to spontaneous chemical oxidation, if allowed to continue without heat dissipation, results in spontaneous ignition at about 280°C.

Replicability of Spontaneous Heating Studies

The heating and respiratory phenomena exhibited by duplicate samples of damp soybeans were studied on completion of a second adiabatic apparatus which was put into operation following the first heating experiment. For this study, two one-pint Dewar flasks and Illini seed samples of 250 g were used, in order to assure adequate aeration at the 2,000 ml daily rate through the entire course of the trial. The soybean sample was conditioned to 18.4% moisture (85% relative humidity) over only a 24-hour period as contrasted to the three-day conditioning period used in the previous trial.

The data shown graphically in Figure 3 followed closely the pattern of respiratory and heating phenomena shown in the first trial, and demonstrated that excellent replication is possible. This experiment revealed that when soybean seeds are placed into respirometer bottles rather promptly after conditioning, a respiration lag period is shown. In addition, the phenomenon of high initial respiratory quotient, which Milner and Geddes (1945) found was associated with the release of carbon dioxide from freshly wetted soybeans, appears.

Influence of Heavy Inocula of Microflora on the Heating and Respiration of Normal Soybeans

To study the influence of heavy inocula of microflora on the spontaneous heating of normal seeds showing low microfloral contamination, a 250-g portion of the high quality Wisconsin Manchu seed was conditioned with sterile water to 18.0% moisture (84% relative humidity)

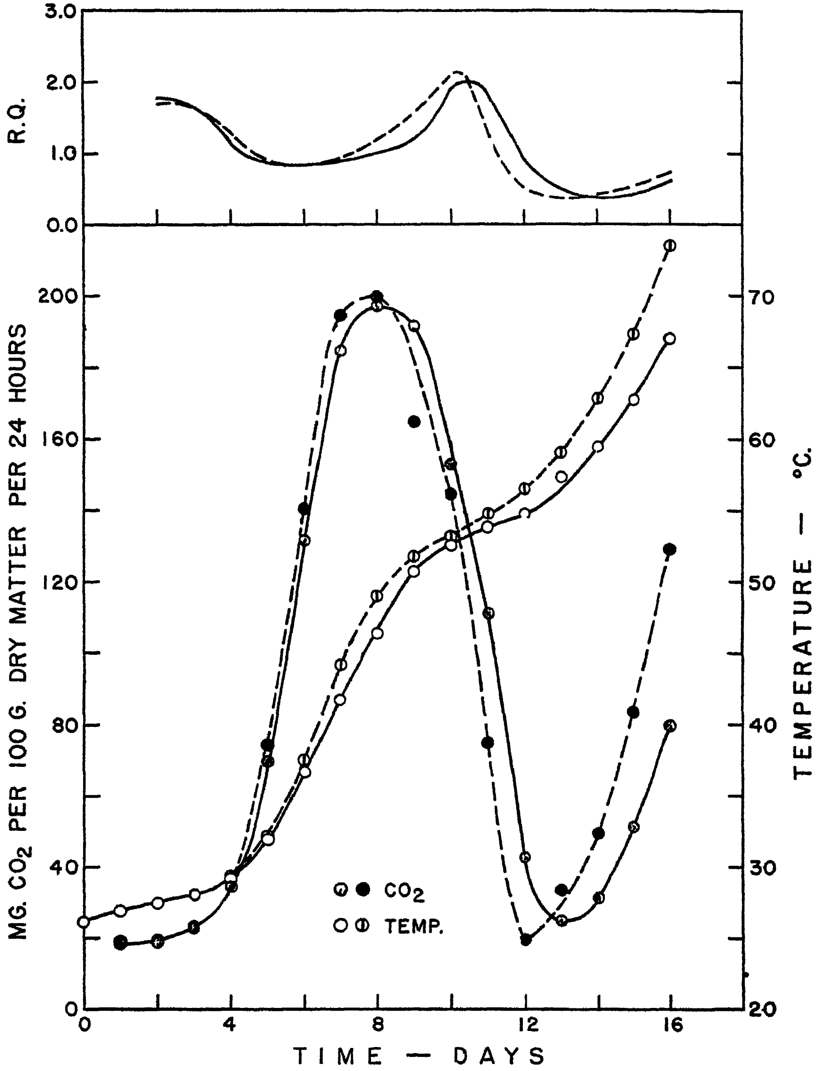


Fig. 3. The replicability of spontaneous, adiabatic heating studies with Illini soybeans at 18.4% moisture.

while a similar subsample received the same volume of an aqueous suspension of microflora representative of the variety of species present in the normal seed, which had been previously isolated from soybeans and propagated in pure culture. The inoculum included spores of *Aspergillus flavus*, *A. ochraceus*, *A. glaucus*, *A. fumigatus*, a *Penicillium* spp., a brown Aspergillus isolated from soybean meal, as well as broth cultures of unidentified bacteria from discolored soybean cotyledons, *Bacillus mesentericus*, and unidentified rod-shaped bacteria obtained from soybeans used in the previous study. Smaller portions of both seed lots in small bottles were placed in the respective air baths, and independently aerated as in the first trial.

As indicated in Figure 4, both the inoculated and noninoculated seed showed very slow initial heating; significant increases in tempera-

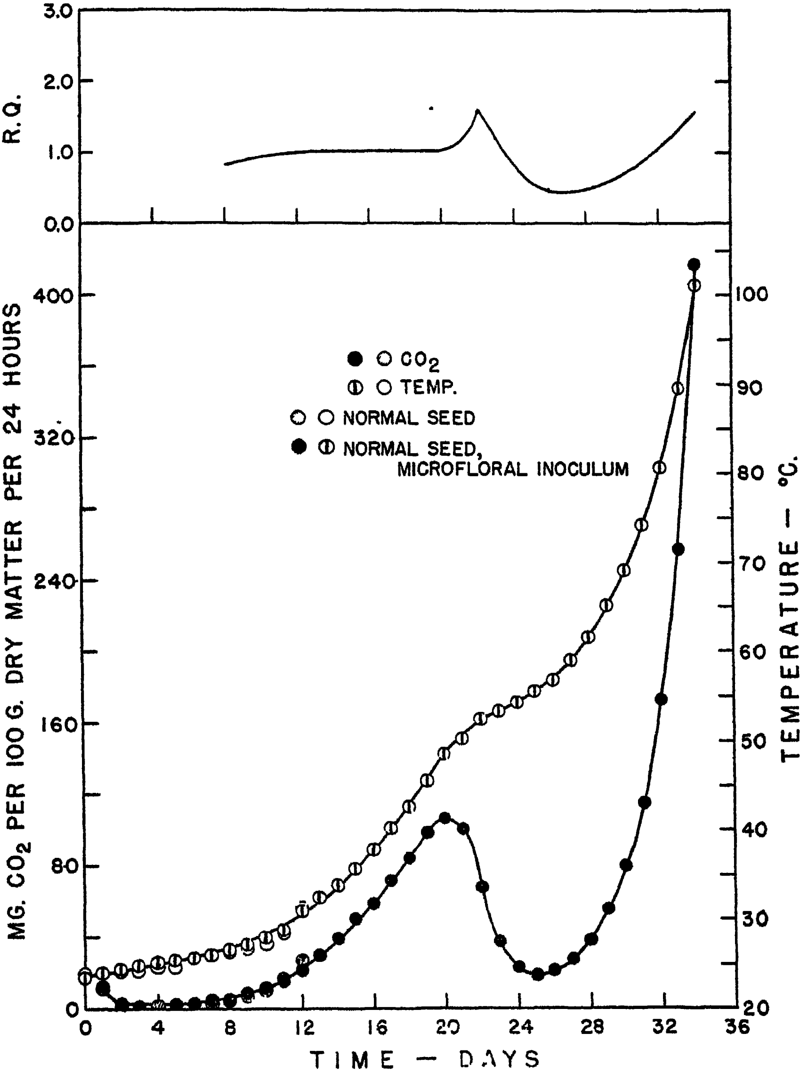


Fig. 4. The spontaneous adiabatic heating and respiratory characteristics of Wisconsin Manchu soybeans at 18.0% moisture and of the same seed heavily inoculated with mixed microflora. The trial for the noninoculated seed was discontinued on the 12th day. The curve for the respiratory quotients applies to the inoculated sample.

ture and respiration occurred only after the eighth day. From this time to the 12th day the course of the heating and respiration was virtually identical for both. The probable reason for this common trend became apparent when on the 11th day, subsamples from both lots were removed and the seeds were cultured in sterile petri dishes. Both the inoculated and noninoculated seed gave rise to a grey-green Aspergillus tentatively identified as *A. glaucus.*

Unfortunately the control device for the adiabatic respirometer containing the noninoculated sample failed on the 13th day. The samples of seed removed from both respirometers at this time were 100% contaminated with the grey-green mold previously noted. The data for the 13-day period showed the inoculated and noninoculated samples to be heating and respiring at virtually the same rate, and it seems quite probable that both samples would have continued to heat and respire in a similar manner thereafter. It was clear that the noninoculated sample, which in a dry state before conditioning showed only 5% of the seeds to be infected by *A. glaucus*, quickly developed 100% infection by this fungus when conditioned and held in the adiabatic apparatus.

Study of the inoculated sample was continued for 34 days, by which time the heating was progressing so rapidly that in the 24-hour interval between the 33rd and 34th days the temperature increased 11.5°C. The temperature at this time was 101°C and was increasing at a rapidly accelerating pace. Marked distillation of water from the system was underway while carbon dioxide concentration in the interseed air had risen to 21%. Increased aeration could not be provided. In view of these factors and the nonfire resistant construction of the apparatus, it was deemed hazardous to carry the study to higher temperatures. There seems little question that if adequate aeration could have been provided, ignition temperatures would have been reached in a few days.

Continued microfloral examination of the seeds in the course of the trial revealed that the grey-green mold *A. glaucus* proliferated more readily than all other mold species in the inoculum. Thus by the 17th day when the temperature was 40.1°C, the seeds showed 100% infection by molds, 95% being *A. glaucus.* On the 19th day (temperature 45.6°C) the only mold arising from the seeds, still 100% infected, was *A. glaucus.* On the 21st day (51°C) no viable molds were found.

Bacterial infection, consisting mainly of *B. mesentericus*, decreased in terms of the percentage of seeds infected following the 17th day, but persisted on 15% of the seeds to the 32nd day when the temperature was 80°C. At the end of the trial (101°C) all the seeds were sterile. It must be stressed that no estimate was made of the *number* of bacterial cells. However, no visual evidence of bacterial proliferation on the samples was noted at any time, in contrast with the heavy mycelial growth shown by the molds prior to thermal killing. In a subsequent experiment, six unidentified bacterial species, which were isolated from the interior tissues of soybeans and grown in pure culture, were used to inoculate autoclave-sterilized soybeans containing 21% moisture (90% relative humidity at 25°C). No heating or respiration attributable to bacterial growth at this humidity level was noted.

Certain points of difference in the respiration and heating characteristics of the Illini and Wisconsin Manchu soybean seeds are to be noted. The Wisconsin Manchu seed showed a very long lag period both for heating and carbon dioxide production as compared to the Illini sample used in the previous trial. Similarly, the Manchu seed showed a considerably lower maximum biological carbon dioxide production rate (106 mg as compared to 200 mg) at approximately the same moisture value. It might be inferred that this particular sample of high quality Wisconsin Manchu soybeans provided a less hospitable medium for the molds than did the Illini sample.

Influence of Surface Sterilization and Mold Inoculation on the Spontaneous Heating and Respiration of Soybeans

The results of the previous trial showed that even the slight microfloral contamination of untreated normal soybean seeds yielded as rapid mold proliferation, respiration, and heating, as did heavy inocula of microflora. In an attempt to inhibit the growth of the normal seed microflora in order to study seed respiration and heating in the absence of that due to the molds, a lot of the Wisconsin Manchu soybeans was sterilized by shaking in a solution of mercuric chloride (1–1,000) for one-half minute. After decantation the seeds were washed for one minute with sterile water and allowed to drain. After 14 hours in a sealed jar, the moisture value of the seed was 19.7% (equilibrium relative humidity 87%). A 250-g sample was transferred to a sterile Dewar flask and smaller portions were placed into sterile 8-ounce bottles for insertion into the adiabatic respirometers for a respiration and heating trial. Another portion of the same sterilized, conditioned seed was heavily inoculated with spores of a pure culture of *A. flavus* and the respiratory and heating characteristics studied in the other adiabatic respirometer.

The respiration and heating data are presented in Figure 5. The surface-sterilized and surface-sterilized reinoculated seeds heated and respired in a virtually identical manner. As the trial proceeded, both seed lots proved to be contaminated with *A. flavus* and *A. glaucus*, the percentage of seeds infected being similar for both at similar time

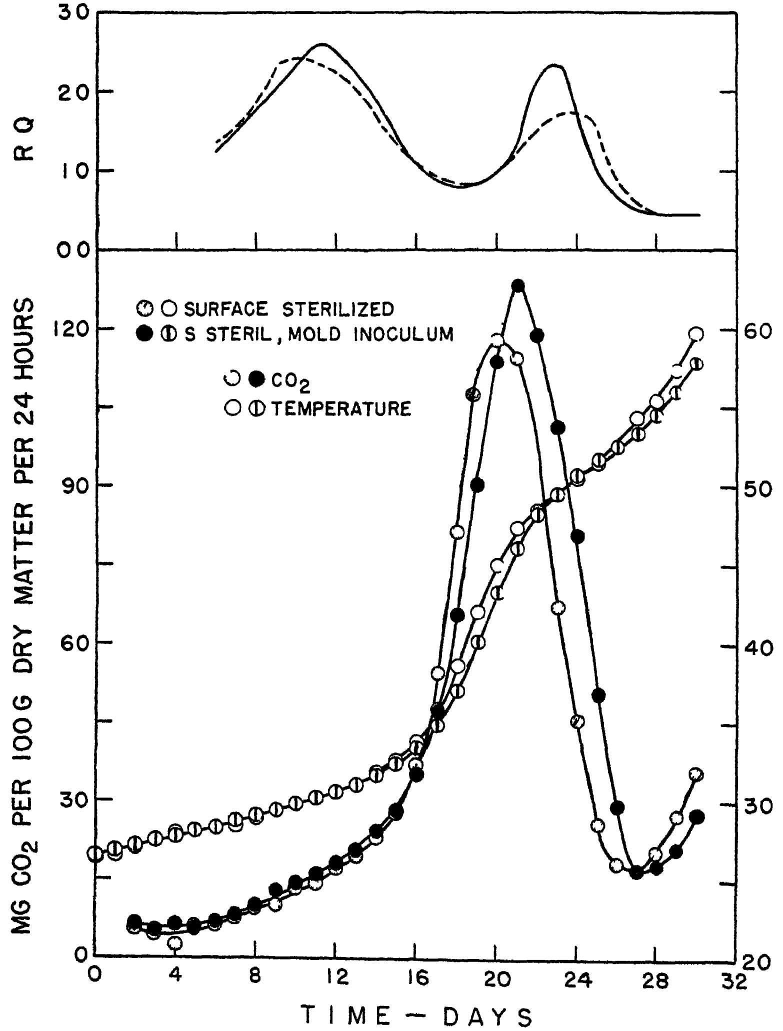


Fig 5 The spontaneous adiabatic heating and respiratory characteristics of surface sterilized Wisconsin Manchu soybeans at 19 7% moisture and of the same seed inoculated with *Aspergillus flavus*

periods. It was apparent that surface sterilization by the technique used was ineffective against mold contaminants, which apparently existed well within the seed coats and were unaffected by sterilization at the surface. This result is in complete agreement with the observation of Mead (1941) who found that seeds treated with mercurial dusts or formalin to kill surface microfloral contaminants would subsequently yield growths of mold, arising from the more deep-seated infections, when cultured on moist media.

Spontaneous Heating and Respiration of Autoclave-Sterilized Soybeans with and without Mold Inoculation

The failure of the surface sterilization technique to provide a means of studying independently the respiration due to molds and seeds suggested a trial involving the inoculation of sterile seeds with molds to obtain data on the respiration and thermogenic behavior of the microorganism itself. It was also of interest to investigate the minimal temperatures at which the secondary spontaneous chemical heating phase would occur.

An adiabatic heating and respiration trial was conducted on a conditioned lot of the Wisconsin Manchu seed which had been autoclaved for one hour at 120°C (together with the requisite Dewar flasks, glass wool-plugged connections, 8-ounce bottles, and other accessories) and then inoculated with spores of a pure culture of *A. flavus*. A subsample from the same lot of autoclaved seeds, but uninoculated, was used in the other adiabatic apparatus. The moisture content of this grain was 20.4% (89% relative humidity).

Samples of both lots of seeds removed from the sterile 8-ounce bottles on the seventh day of trial showed the inoculated seeds to be 100% contaminated with *A. flavus*, while the sterilized noninoculated seeds were completely sterile.

The respiration and heating data for this trial are given in Figure 6. The course of the carbon dioxide evolution, due entirely to the metabolism of the mold *A. flavus*, is almost identical to that given by normal seed where the organism was a natural contaminant. The heating curve shows similar characteristics to that of normal seeds, including the attainment of a plateau resulting from the thermal death of the microflora, which is followed by secondary heating due to chemical oxidation. The curve for the respiratory quotients is somewhat different in that the sharp peak yielded by normal seeds following biological thermal killing is absent. This phenomenon seems to be associated with temperature-induced evolution of carbon dioxide from initially normal seeds, and is therefore not related to the respiratory exchange or the thermal death of the mold population.

The apparent slight heating shown by the sterile seeds in the first 13 days of the trial is ascribed to the calibration anomaly previously discussed. No detectable carbon dioxide evolution appeared in this temperature range (22° to 25°C). To ascertain if chemical heating was possible without initial biological activity, the auxiliary heaters of the thermostat containing the sterile seeds were turned on for a short period, thus raising the temperature of the seeds in the Dewar flask to 31.1°C; the thermocouple control was then reestablished.

Subsequently, similar temperature increases were applied, as shown in Figure 6.

The slight temperature increase beginning at 31.1°C (13th to 21st day) may be accounted for by the calibration anomaly; however, detectable amounts of carbon dioxide in the order of 0.5 mg per 100 g dry matter weight appeared. At 40.5°C (22nd day) no temperature change was recorded over a two-day interval, but the carbon dioxide

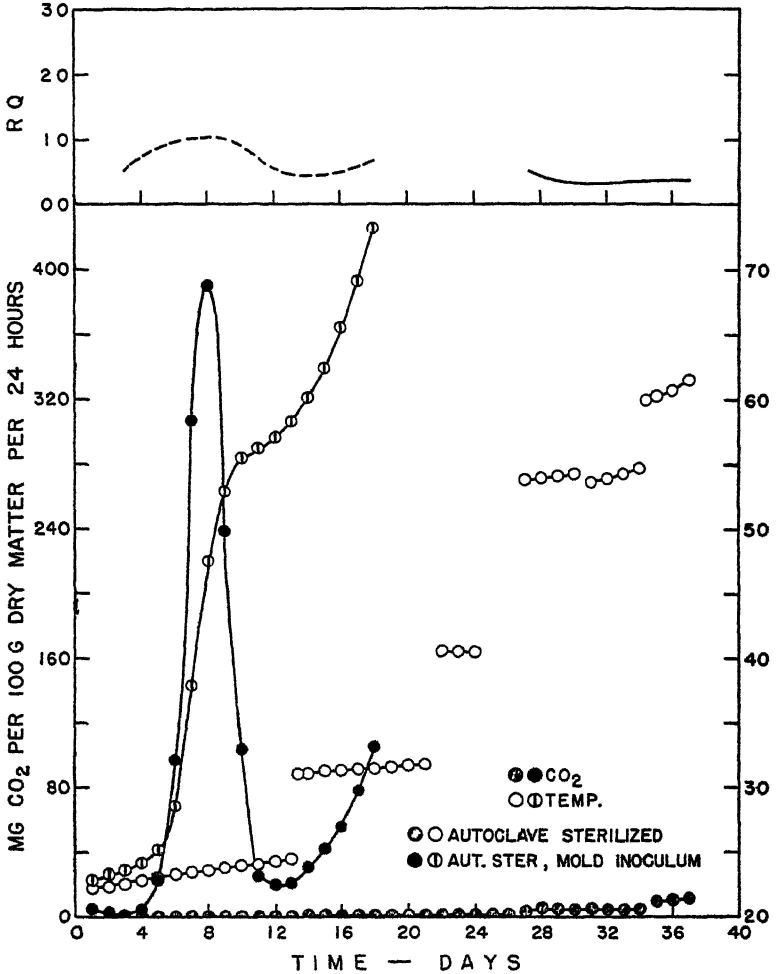


Fig. 6. The spontaneous adiabatic heating and respiratory characteristics of autoclave-sterilized Wisconsin Manchu soybeans at 20.4% moisture, and of the same seed reinoculated with *Aspergillus flavus*. In the latter case, the sample was artificially adjusted to different temperatures after various time intervals and the spontaneous heating at each temperature level determined for several days. The R.Q. curve shown as a dotted line pertains to the mold-inoculated sample; the curve for the sterile sample is shown as a solid line.

production rose to 1.2 mg. The constant temperature during this interval indicated this temperature to be close to the value at which virtually adiabatic calibration conditions existed, and that a balance existed between heat production and loss.

At 53.8°C (27th day) definite spontaneous heating was noted, the carbon dioxide production being 4.7 mg. Even stronger spontaneous heating appeared at 60°C, a temperature where cooling would occur on calibration. The mean carbon dioxide production for the three days following establishment of this temperature was 10.2 mg. The respiratory quotient of this gas exchange was 0.4, corresponding to that shown by normal or autoclaved inoculated seeds in the initial ranges of spontaneous chemical heating, following the thermal inactivation of biological agencies. This experiment demonstrates that carbon dioxide production due solely to chemical oxidation occurs in the temperature range where microorganisms are active, and that spontaneous heating due to chemical oxidation is possible at higher temperatures even without the normal preliminary activity of the microorganisms.

Discussion

Two distinct ranges of temperature increase have been shown to occur with dormant soybeans heating spontaneously under adiabatic conditions at humidity levels sufficiently high as to favor the proliferation of mold fungi, yet too low to cause bacterial growth. The first heating phase, which is a consequence of the respiration and metabolism of the proliferating molds, ends when the thermal death range (50°–55°C) of these microflora is reached. The species *Aspergillus glaucus* and *A. flavus* were primarily involved. The second heating stage, concealed in its lower range under natural conditions by the upper temperature range achieved by the molds, appears clearly at temperatures higher than the thermal death range of the molds and seeds. The fact that autoclave-sterilized seeds would heat and produce carbon dioxide spontaneously, in a manner directly similar to normal seeds where preliminary microfloral activity occurs, indicates that this secondary heating cannot be ascribed to enzymic or other biological phenomena. Under adiabatic conditions and with sufficient aeration, this secondary spontaneous heating due to nonbiological oxidation apparently has no upper limit short of the ignition temperature of the seed materials.

The present studies have shown that a close parallelism between rate of heating in terms of temperature increment and carbon dioxide production exists in all stages when adequate aeration is provided. This is evident from a comparison of the daily temperature increments with the respiratory rates.

The inadequacy of surface sterilization techniques in preventing growth of the contaminating mold spores precluded study of seed respiration itself. On the other hand, heating studies conducted by inoculating autoclave-sterilized soybeans with spores of a pure culture of *Aspergillus flavus* showed that the molds are primarily responsible for the characteristic course of spontaneous heating and respiration noted with normal soybeans. Except for certain minor respiratory phenomena, such as a brief initial evolution of carbon dioxide due to wetting of the seed, and a similar dissipation of this gas immediately following the thermal death point of the seeds as evidenced by a sharp peak in the respiratory quotient, the respiration of the dormant seed seems to play virtually no part in the heating. Under practical conditions, other than those where the moisture content would be sufficiently high to cause seed germination, the respiration of the seed itself is insignificant in storage and heating phenomena.

Of the changes in the various chemical factors in the seeds which were assayed at intervals in the course of the heating trials, the most notable was the decrease in the petroleum ether soluble fraction which occurred in the temperature range of spontaneous chemical heating. The data indicate that this loss of oil is only an apparent one since no consequent loss of dry matter weight takes place. This effect is probably due to oxidative and thermal polymerization of the unsaturated seed oils, rendering them less soluble in petroleum ether. This phenomenon may be responsible for the ease with which spontaneous chemical heating occurs in soybeans, following thermal inactivation of the biological thermogenic agencies.

It is speculated that the apparent increase in total reducing substances with the onset of chemical heating may be due to thermal cleavage of carbohydrate molecules to yield numerous smaller reducing fragments of high oxygen-absorbing capacity. Such carbohydrate degradation products might be classed with the pyrophoric compounds whose formation as a preliminary to spontaneous combustion has been postulated by Miehe (1911) and Browne (1929).

Summary

Spontaneous heating and associated respiratory characteristics of soybeans at humidity levels favorable to mold growth but unfavorable to bacterial proliferation were studied in an apparatus which maintained continuously controlled adiabatic and aeration conditions, over time intervals up to 37 days. An initial temperature increase to 50°–55°C and parallel respiratory increase were directly associated with the proliferation of the molds *Aspergillus glaucus* and *A. flavus*.

A secondary spontaneous heating phase due to nonbiological oxida-

tion was demonstrated, which appeared to have no limit short of ignition temperatures, and was closely paralleled by the extent of carbon dioxide evolution.

Surface sterilization of the seeds failed to eliminate mold infection, whereas inoculation of autoclave-sterilized soybeans with spores of *Aspergillus flavus* yielded heating and respiration curves virtually identical to those of normal seeds.

Spontaneous heating of sterile seeds in which no microfloral activity had occurred was demonstrated. Chemical changes in the heating seeds assayed at intervals in the course of the trials indicated a disappearance of total sugars in the initial biological phase of heating and an increase in reducing substances in the initial spontaneous chemical heating phase. The petroleum ether-soluble fraction remained virtually unchanged in the biological heating stage but decreased markedly in the chemical heating phase, without a corresponding loss in dry matter content of the seeds.

Respiratory quotients associated with the gas exchange during the spontaneous chemical heating phase suggest the occurrence of thermally induced oxidative cleavage of carbohydrates as well as oxidative polymerization of the seed oils.

Acknowledgment

The authors are indebted to Mr. E. A. Andrews and Dr. C. M. Christensen, of the Division of Plant Pathology, University of Minnesota, who provided the mycological data.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods (Fourth Ed.). American Association of Cereal Chemists, Lincoln, Neb.
Bailey, C. H.
1940 Respiration of cereal grains and flaxseed. Plant Physiol. **15**: 257–274.
——, and Gurjar, A. M.
1918 Respiration of stored wheat. J. Agr. Research **12**: 685–713.
Bakke, A. L., and Henson, E. R.
1933 Relation of fungi to respiration and fermentation occurring in stored hay Iowa Agr. Expt. Sta. Report on Agr. Research, p. 51.
——, and Noecker, N. L.
1933 The relation of moisture to respiration and heating in stored oats. Iowa Agr. Expt. Sta. Research Bull. **165**: 319–336.
Becker, H. C., Milner, R. T., and Nagel, R. H.
1940 A method for the determination of non-protein nitrogen in soybean meal. Cereal Chem. **17**: 447–457.
Browne, C. A.
1929 The spontaneous combustion of hay. U.S.D.A. Tech. Bull. 141.
1933 The spontaneous heating and ignition of hay and other agricultural products. Science **77**: 223–229.
Carlyle, R. E.
1940 Measurement of the heat liberated in the biological decomposition of plant materials. Iowa State Coll. J. Sci. **15**: 60–62.
——, and Norman, A. G.
1941 Microbial thermogenesis in the decomposition of plant materials. Part II. Factors involved. J. Bact. **41**: 699–724.

Cohn, F.

1890 Ueber Warme Erzeugung durch Schimmelpilze und Bakterien. Jahresber. Schles. Gesell. (Breslau) 68: 23–29. Cited by Browne (1929). (Original not seen.)

Darsie, M. L., Elliot, C., and Peirce, G. J.

1914 A study of the germinating power of seeds. Bot. Gaz. 58: 101–136.

Gilman, J. C., and Barron, D. H.

1930 Effect of molds on temperature of stored grain. Plant Physiol. 5: 565–573.

Haldane, J. S., and Makgill, R. H.

1934 The spontaneous oxidation of coal and other organic substances. J. Soc. Chem. Ind. 53: 359–367 T.

Harrison, J. W.

1934 Thermogenesis in hay inhabiting fungi. Iowa State Coll. J. Science 9: 37–60.

Hoffman, E. J.

1935 Oxidation and gas formation in the spontaneous heating of hay. J. Agr. Research 51: 527–546.

1940 Thermal decomposition of undercured alfalfa hay in its relation to spontaneous ignition. J. Agr. Research 61: 241–257.

James, L. H.

1927 Studies in microbial thermogenesis. I. Apparatus. Science 65: 504–506.

——, Rettger, L. F., and Thom, C.

1928 Microbial thermogenesis. II. Heat production in moist organic materials with special reference to the part played by microorganisms. J. Bact. 15: 117–141.

Mead, H. W.

1941 The disinfecting value of fungicides used for treating cereal seeds and their influence on growth. Sci. Agr. 21: 717–726.

Miehe, H.

1911 Ueber die selbsterhitzung des Heues. Arb. deut. landw. Gesell. 196: 1–47. Cited by Hoffman (1935). (Original not seen.)

Milner, M., Warshowsky, B., Tervet, I. W., and Geddes, W. F.

1943 The viability, chemical composition and internal microflora of frost damaged soybeans. Oil and Soap 20: 265–268.

——, and Geddes, W. F.

1945 Grain storage studies. II. The effect of aeration, temperature and time on the respiration of soybeans containing excessive moisture. Cereal Chem. 22: 484–501.

1946 Grain storage studies. III. The relation between moisture content, mold growth, and respiration of soybeans. Cereal Chem. 23: 225–247.

Norman, A. G., Richards, L. A., and Carlyle, R. E.

1941 Microbial thermogenesis in the decomposition of plant materials. Part I. An adiabatic fermentation apparatus. J. Bact. 41: 689–697.

Ramstad, P. E., and Geddes, W. F.

1942 The respiration and storage behavior of soybeans. Minn. Agr. Expt. Sta. Tech. Bull. 156.

Sallans, H. R., Sinclair, G. D., and Larmour, R. K.

1944 The spontaneous heating of flaxseed and sunflower seed stored under adiabatic conditions. Can. J. Research F22: 181–190.

Wedberg, S. E., and Rettger, L. F.

1941 Factors influencing microbial thermogenesis. J. Bact. 41: 725–743.

THE EFFECT OF PRESSURE ON MACARONI DISCS: SIZE AND NUMBER OF AIR BUBBLES IN RELATION TO LIGHT TRANSMISSION[1]

GLENN S. SMITH,[2] R. H. HARRIS,[3] ETHEL JESPERSON,[4]
and L. D. SIBBITT[5]

(Received for publication April 17, 1946)

All macaroni products normally contain air bubbles. Air is introduced in the process of mixing the doughs, but bubbles cannot be found on the surface of macaroni products, perhaps because of surface tension. Microscopic examination of the fractured surfaces, however, reveals numerous bubbles, 200 to 400 μ in diameter. This paper presents evidence that the size and number of these air bubbles are related to the translucency of the finished macaroni.

Translucency is very important in macaroni products. The endosperm of a vitreous wheat kernel is translucent (Alsberg, 1939), and even individual starch grains under the microscope are translucent. However, the appearance of translucency to the naked eye is lost in processing the semolina and not recovered until pressure is applied to the dough.

The significance of pressure in developing translucency was pointed out by Fifield, Smith, and Hayes (1937) in describing the macaroni disc method, a technique proposed for studying the quality of small samples of durum. Cunningham and Anderson (1943a) studied the effects of processing conditions on paste properties of macaroni discs as measured by the optical coefficient of opacity. They concluded that pressing, mixing, and absorption had the greatest influence on opacity (or translucency). The latter workers used bubble size in determining the pressure needed to yield an internal structure comparable with that of commercial macaroni products. They suggested that longer mixing time increases opacity chiefly because it increases the number of minute air bubbles incorporated into the paste.

Before pressing, macaroni dough is dull in color, resembling putty, and the first few inches of the extruded product from the die are still white in color and opaque. Gradually, however, as more material is extruded this color becomes clear and translucent. Microscopic examination of the internal structure of this macaroni shows that the opaque section is honeycombed with numerous small bubbles, while the translucent section contains relatively few large bubbles. This observation reveals the significance of time as well as pressure in relation to translucency of the finished product. Accordingly, the experiments reported in this paper were conducted to determine the effects of varying both intensity and duration of pressure upon translucency and associated bubble size and number. A third variable, durum variety, was introduced because of the importance of color characteristics in breeding new varieties.

---

[1] Joint contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Cereal Technology, North Dakota Agricultural Experiment Station.

[2] Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, field headquarters at Fargo, North Dakota.

[3] Cereal Technologist, North Dakota Agricultural Experiment Station.

[4] Research Assistant, Department of Cereal Technology, North Dakota Agricultural Experiment Station.

[5] Assistant Cereal Technologist, North Dakota Agricultural Experiment Station.

Materials and Methods

The semolinas used in this study represented six varieties of durum grown in the 1943 wheat variety field plots at Langdon, North Dakota. They ranked from good to poor in quality in the following order: Carleton, Mindum, Stewart, Ld. 153, Kubanka-314, and Monad.

In the experimental method of processing tube macaroni (Fifield, 1934; Binnington and Geddes, 1936; and Harris and Sibbitt, 1942) it is not feasible to vary pressure because the dough is constantly changing in volume. Furthermore, the shape of conventional macaroni products is not suitable for objective measurements of light transmission and the true internal structure is masked by the roughened surface caused by friction with the die. On the other hand, the macaroni disc method of Fifield *et al.*, 1937 (the "micro test" of Cunningham and Anderson, 1943) is much more suitable for experimental study of the fundamental factors involved in macaroni manufacture. Any range of pressure or time can be applied, dough volume is approximately constant, the thin disc is convenient for measurements of color characteristics or light transmission, and the surface is not modified by friction.

Equipment available for these experiments was similar to that described by Fifield *et al.* (1937). Procedure in general was as follows: 30 g of semolina was used with 30% absorption and 120 revolutions of the mixer, followed by sheeting 30 times. Two discs were pressed from each sample. Drying was judged complete after two days between smooth faces of corrugated paper. Four pressures, 1,250, 1,500, 2,000, and 3,000 pounds per square inch for time periods of 20, 60, and 240 seconds, were chosen after preliminary experiments to determine what combinations would give the desired ranges in translucency. A lower range gauge would have been preferable to give more sensitive readings at still lower pressures, but at the time the work was undertaken only the 10-ton gauge was available.

Bubble numbers and diameters were determined under a microscope with an eyepiece micrometer at magnifications ranging from 17 to 430 diameters, as required by the various sizes of bubbles. Ten readings were made for each of the twin discs. Bubble counts were calculated to the number per 10 squares in the eyepiece reticule at 17 ×, which actually represented 6 94 square millimeters. Under the microscope it was possible to take bubble counts only in two dimensions. The square root of the number thus counted corresponds to the cube root of the number present in three dimensions To facilitate graphing the data, bubble number was tabulated and shown in terms of the cube root of the number present in three dimensions Diameter of bubbles was calculated to microns Translucency of the discs, or the amount of transmitted light that could pass through them, was measured with a photo-electric cell in terms of galvanometer scale units. The photo-electric cell assembly is illustrated in Figure 1. It consisted of a photronic cell wired to a galvanometer with a resistance box connected in parallel. The cell was placed facing the light source at a distance of 12 inches. The aperture of the cell was smaller than the discs to be measured. The most translucent macaroni disc was first held over the cell, and the resistance adjusted to give a maximum reading on the galvanometer. All discs were then read at this resistance. The light source was a small laboratory spotlight using a 100 watt bulb.

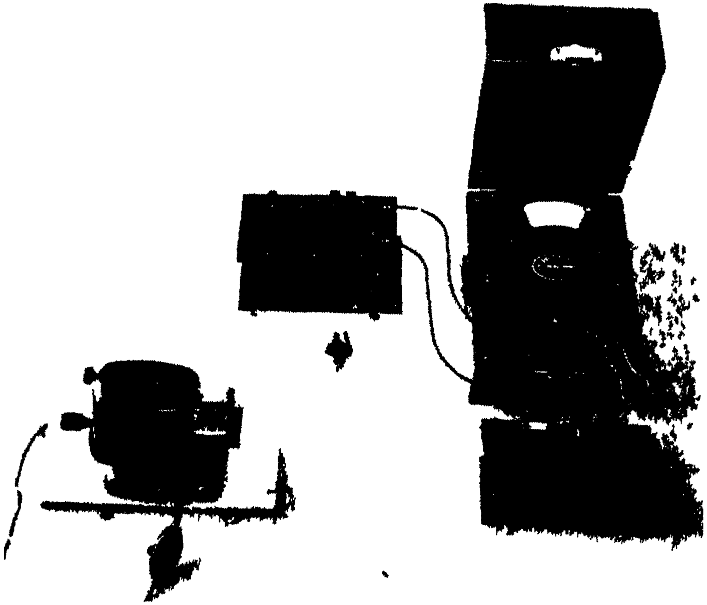


Fig 1 Photo electric cell assembly for measuring light transmission of macaroni discs

Experimental Results

Increasing duration and intensity of pressure on the macaroni discs caused the minute air bubbles to coalesce, reducing their number, thus increasing their size and augmenting the translucency of the discs.

Number of Bubbles. Table I presents data on the (cube root of) average bubble number for the six varieties at the various pressure and time period treatments.

TABLE I

EFFECT OF PRESSURE APPLIED FOR VARIOUS TIMES ON AVERAGE BUBBLE NUMBERS

Pressure	Time	Average bubble numbers [1]						
		Carleton	Ld. 153	Mindum	Stewart	Monad	Kubanka	Average
lb	*sec*							
none	none	77.8	67.4	70.4	70.4	66.1	65.6	69.6
1,250	20	23.0	23.9	23.5	23.2	24.2	23.5	23.6
1,500	20	9.7	10.0	10.1	9.6	10.2	9.7	9.7
2,000	20	4.7	4.9	4.6	4.7	4.7	4.6	4.7
3,000	20	3.1	3.5	2.7	3.0	3.4	3.1	3.1
1,250	60	14.1	13.9	12.8	14.3	13.8	13.5	13.7
1,500	60	4.6	5.7	4.3	5.1	4.5	4.3	4.8
2,000	60	3.5	3.9	3.6	3.6	3.2	3.4	3.5
3,000	60	2.4	2.8	2.9	2.7	2.3	2.3	2.6
1,250	240	4.0	5.1	4.4	4.0	3.8	3.4	4.1
1,500	240	3.2	3.4	3.2	3.0	3.0	2.6	3.1
2,000	240	2.9	2.9	3.0	2.7	2.3	2.7	2.8
3,000	240	2.1	2.2	2.4	2.1	2.1	1.7	2.1
Varietal averages		11.7	11.5	11.4	11.4	11.0	10.8	11.3

[1] Bubble numbers are expressed as cube root of average number determined in a disc volume of 18.5 cubic millimeters, 10 counts in each of twin discs for each variety treatment.

The relation between average number of bubbles and the pressure applied to the discs is curvilinear. The curves have been drawn to show an initial (dotted section) lag which was clearly evident when using pressures below 1,250 pounds as registered by a low pressure gauge in supplementary experiments. This is interpreted as the "yield value" (Gortner, 1938) characteristic of all plastic systems. After the lag period is overcome, the number of bubbles decreases greatly with relatively small increments of pressure. This raises the question whether the air bubbles are squeezed out, or merely coalesced into larger and fewer bubbles. Data presented later on the size of bubbles give no indication that any air is forced out of the dough.

The effect of pressure increases with the length of time it is applied, for example 2,000 pounds at 20 seconds, 1,500 pounds at one minute,

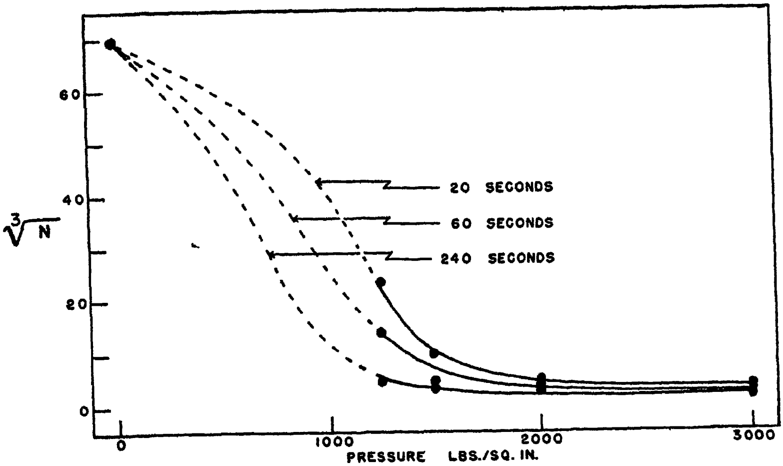


Fig. 2. Average number of air bubbles (expressed as the cube root) in macaroni discs made from six durum wheat varieties, employing various pressures for different time intervals. Under the microscope it was possible to take bubble counts in only two dimensions. The square root of the number thus counted corresponds to the cube root of the number present in three dimensions.

and 1,250 pounds at four minutes all gave approximately the same number of bubbles. The actual number of bubbles in a given volume in the unpressed check was nearly 40,000 times that in the disc receiving 3,000 pounds pressure for four minutes. Varietal differences in bubble number were small.

TABLE II

Effect of Pressure Applied for Various Times on Average Diameter of Air Bubbles

Pressure	Time	Average bubble diameters						
		Ld. 153	Mindum	Carleton	Stewart	Monad	Kubanka	Average
lb	*sec*	*μ*	*μ*	*μ*	*μ*	*μ*	*μ*	*μ*
none	none	11	17	14	13	13	12	13.4
1,250	20	24	27	28	30	31	34	29.0
1,500	20	54	86	78	69	62	85	72.5
2,000	20	130	151	165	155	209	186	166.0
3,000	20	264	326	273	304	293	312	295.0
1,250	60	62	59	60	56	58	63	59.8
1,500	60	165	190	194	207	226	245	204.6
2,000	60	272	253	309	268	356	293	291.9
3,000	60	338	332	380	419	479	437	397.6
1,250	240	184	210	245	236	260	318	233.7
1,500	240	289	267	277	347	416	394	348.2
2,000	240	319	323	341	400	425	401	368.4
3,000	240	334	421	417	522	611	673	496.2
Varietal averages		203	220	231	251	286	295	247.6

Size of Bubbles. Macaroni bubbles in commercial macaroni products are highly variable in size, but often range from 200 to 400 μ in diameter Average bubble diameters in microns for the various treatments and varieties are given in Table II and the treatment averages are shown in Figure 3. The initial lag period is more clearly indicated by these data than in the case of bubble numbers. The effect of duration of pressure is more strikingly illustrated at the lowest pressure, 1,250 pounds. At 20 seconds the diameter of the bubbles was barely double that of the check, at one minute it had increased four times, but at four minutes the diameter had increased nearly 20 times. Time was relatively less important as pressures were increased. At 3,000 pounds pressure, 20 seconds of pressing was sufficient to produce larger bubbles than four minutes at 1,250 pounds.

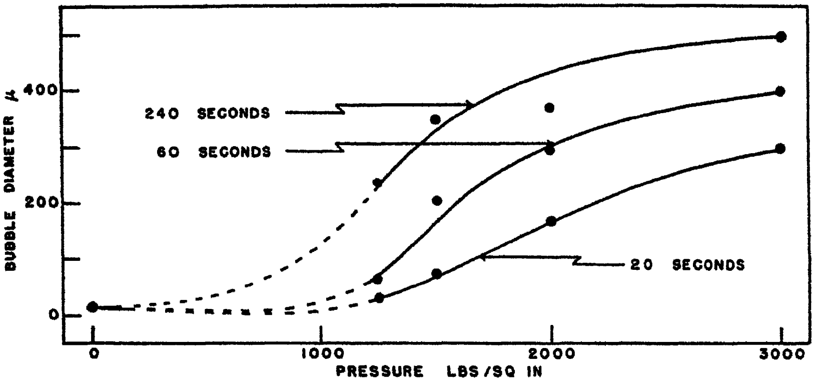


Fig 3 Average size of bubbles in macaroni discs made from six durum wheat varieties, employing various pressures for different time intervals

In the case of bubble size, the curves had not leveled off at 3,000 pounds of pressure for four minutes. Although this treatment gave bubbles 40 times as large in diameter as the checks, it appears that either higher pressures or longer time periods would have given still larger bubbles.

Photomicrographs showing the sizes of bubbles at four selected treatments are given in Figure 4.

The effect of duration of pressure on macaroni products is less obvious than the effect of intensity of pressure alone. It was previously mentioned that the first macaroni pressed out of the die is opaque, but that subsequent portions gradually become translucent. Actual measurements of size of bubbles in such a tube of macaroni gave as an average of 10 determinations each, 65 ± 0.08 μ for the opaque section, and 309 ± 0.21 μ for the translucent section. Both sections of the macaroni tube had received pressure sufficient to force them through the die, but the high pressure had not been acting on the opaque section long enough to make it translucent

Light Transmission of Discs. Light transmission of the various discs is given in Table III and Figure 5. The curves resemble those for bubble number and bubble size. First there is a definite lag period, then a rapid increase in light transmission at the same treatments which gave rapid changes in bubble numbers and bubble size The light transmission curves tend to level off more rapidly than did those representing bubble size, presumably toward a value determined by the index of transmission of the pure macaroni without any bubbles.

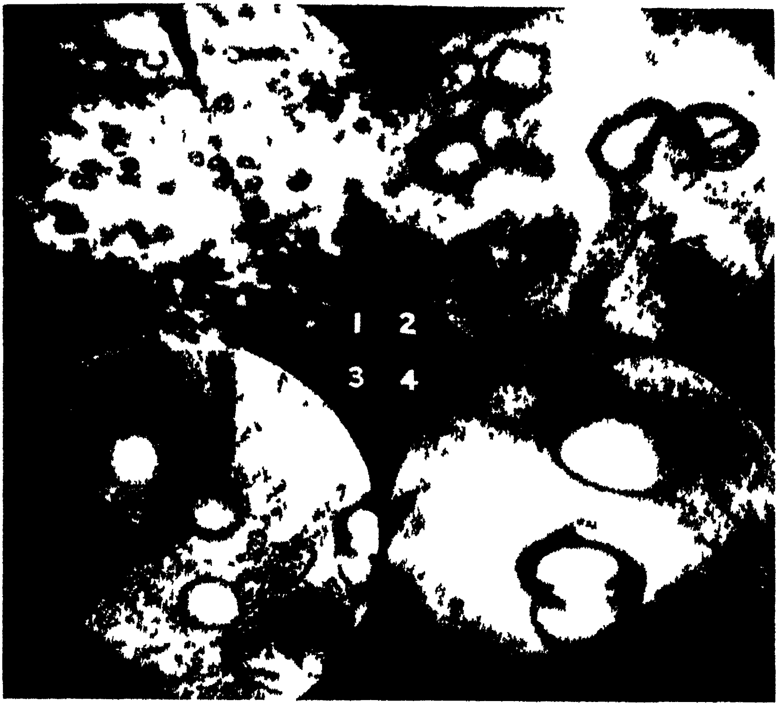


Fig 4 Photomicrographs showing relative size of bubbles in selected macaroni discs (1) 1 500 pounds for 20 seconds, (2) 3 000 pounds for 20 seconds, (3) 1 500 pounds for 240 seconds, (4) 3,000 pounds for 240 seconds

Changes in light transmission were very striking. The most severe treatment permitted the passage of seven times as much light as the unpressed checks. These values give quantitative expression to the observation that "translucency is a result of pressure."

Analyses of Variance. The variance analyses are summarized in Table IV for the different variables investigated. Values determined

TABLE III

EFFECT OF PRESSURE APPLIED FOR VARIOUS TIMES ON AVERAGE LIGHT TRANSMISSION OF MACARONI DISCS

Pressure	Time	Light transmission values [1]						
		Ld. 153	Monad	Mindum	Kubanka	Stewart	Carleton	Average
lb	*sec*							
none	none	3.2	2.9	2.6	4.9	2.8	4.5	3.4
1,250	20	7.6	7.4	6.9	9.1	7.3	7.1	7.6
1,500	20	10.6	11.0	12.4	9.7	11.3	10.1	10.9
2,000	20	16.6	17.4	18.2	17.9	18.0	17.7	17.6
3,000	20	19.4	19.8	20.5	19.8	20.7	21.2	20.2
1,250	60	11.6	11.1	10.3	11.3	11.2	11.9	11.2
1,500	60	17.2	16.7	18.5	17.1	17.2	18.4	17.5
2,000	60	20.8	20.1	19.5	20.8	20.5	22.4	20.5
3,000	60	21.8	22.4	22.6	22.5	21.6	23.7	22.4
1,250	240	18.8	18.9	19.4	19.2	19.2	20.1	19.3
1,500	240	20.1	20.5	21.0	20.9	22.5	20.6	20.9
2,000	240	20.7	21.5	21.9	21.8	23.3	22.0	21.9
3,000	240	22.4	24.4	23.3	22.7	24.1	24.6	23.6
Varietal averages		16.2	16.5	16.7	16.7	16.9	17.2	16.7

[1] Light transmission measured in galvanometer scale units at 1.7 ohms resistance.

for twin discs at each treatment entered separately into the calculations, to give a basis for evaluating experimental error. Data for the unpressed checks were not included in the analyses.

In the case of bubble numbers, both time and pressure, and their interaction, made highly significant contributions to variance, but

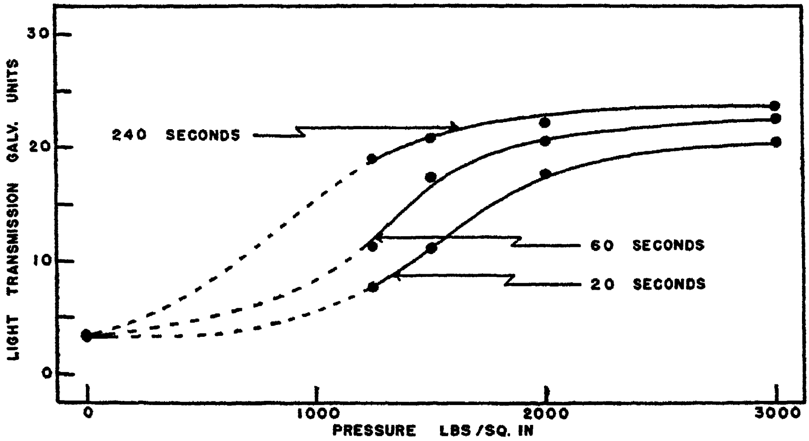


Fig. 5. Average light transmission of macaroni discs made from six durum wheat varieties, employing various pressures for different time intervals.

TABLE IV

VARIANCE ANALYSES OF BUBBLE NUMBER, BUBBLE SIZE, AND LIGHT TRANSMISSION

(Data for unpressed checks not included)

Variance due to	Degrees of freedom	Mean squares for		
		Bubble number	Bubble size	Transmission
Varieties	5	4.0	32,324 **	31.0 **
Time	2	833.5 **	599,793 **	6,479.0 **
Pressure	3	1,126.7 **	519,456 **	6,164.0 **
Varieties × time	10	3.7	10,042 *	16.5 *
Varieties × pressure	15	2.5	3,497 *	13.1 *
Time × pressure	6	210.3 **	11,284 **	631.7 **
Error (V × T × P)	30	1.9	1,519	6.7
Total (Treatments)	71			
Twins (Within treatments)	72	0.3	89	0.9
Total	143			

* Significance exceeds 5% point.
** Significance exceeds 1% point.

neither varietal differences nor the interactions involving variety showed statistical significance.

All three variables, and their first order interactions, contributed very significantly to the variability of both bubble size and light transmission, although variety was consistently least important.

Analysis of the variance in bubble number, bubble size, and light transmission values clearly showed in each case that the time and pressure treatments were mainly responsible for the differences.

Discussion

If no air is forced out, one would expect the number of bubbles to vary inversely with the cube of the diameter. In Figure 6, bubble diameter is plotted against bubble number for each treatment. This gives a hyperbolic curve characteristic of the reciprocal relation, expressed by the equation

$$xy = a$$

where a is a constant, x is the diameter of the bubbles, and y the cube root of bubble number. The data give a good fit to the theoretical curve for this function.

Using logarithms of x and y, the relation may be transformed to a straight line. Figure 6 shows that the fit of the transformed data to the straight line is very good.

Size of bubbles at the various treatments, therefore, corresponds closely with the size calculated from the corresponding bubble numbers.

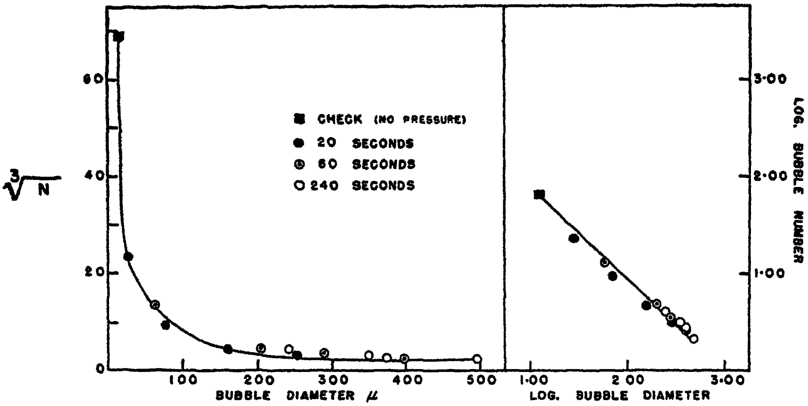


Fig. 6. The relation of bubble diameter to cube root of bubble number ($\rho_{yx} = 0.96$), and of log bubble diameter to log bubble number in macaroni discs made with varying pressures applied for different time intervals ($r_{yx} = -0.99$).

Plotting light transmission of the discs against the corresponding bubble size gave the graph shown in Figure 7. The plotted points follow a curvilinear path, resembling a logarithmic curve. Plotting light transmission against the logarithm of the bubble diameter gave the straight line shown in Figure 7. By inspection, the following formula for the straight line was derived:

$$Y = 13.8 \log x - 13.8$$

where Y is light transmission and x is bubble diameter. Transforming $\log x$ to the antilog, the theoretical curve was then drawn, and it fitted the original plotted points very satisfactorily.

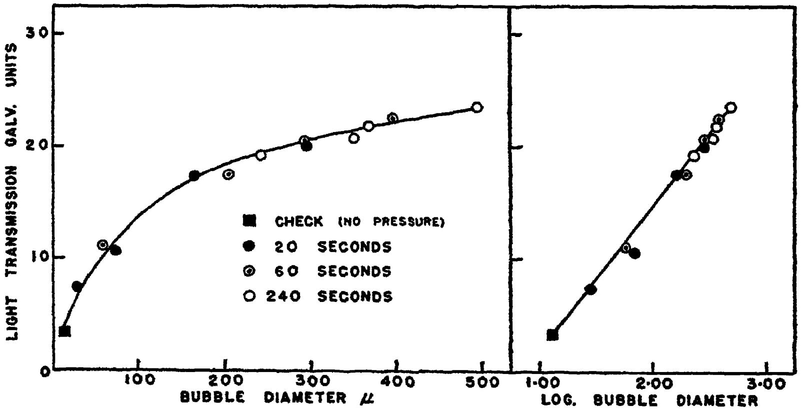


Fig. 7. The relation of light transmission to bubble diameter, and to log bubble diameter in macaroni discs made with varying pressures applied for different times.

The data did not fit a parabolic curve of the type:

$$y^2 = 2\,px.$$

The above curve does not represent the inherent effect of bubble size alone upon light transmission. When dry macaroni products or macaroni discs are held up to the light, the bubbles are apparent as shadows. Therefore, increasing bubble diameter, of itself, would reduce transmission, were it not for the simultaneous decrease in bubble number. Bubble number changes inversely as the cube of the diameter and the curve indicates that the net change in transmission is in the direction of change in number. In this experiment, bubble diameters increased 40 times when the maximum pressure was applied, but simultaneously bubble numbers were reduced 40,000 times, so translucency is affected most by the latter. The curve represents the resultant effect of the two opposing influences, bubble size and bubble number, in a closed system where decrease in number involves an increase in size.

Variations in light transmission were plotted against bubble numbers also, but it was more difficult to reduce this curve to a mathematical formula; hence, it is not presented here. The same interacting effects are involved as discussed above. Light entering the unpressed disc is so diffused by the numerous bubbles that most of it cannot pass through; whereas, the light passes through the pressed discs with only slight interruption by the few bubbles, even though these are relatively large.

One may speculate as to what causes the bubbles to coalesce under pressure. Theoretical considerations suggest that in coalescing, the bubbles are reducing their total surface area exposed to pressure. Eight bubbles, each of one unit diameter, would reduce their total surface area one-half by coalescing into a single bubble of two units diameter. Bubbles within the plastic dough are more or less free to move, and in coalescing they reduce the total pressure on the enclosed air.

However, this appears to be a result rather than the cause of coalescing. As a cause, it is suggested that the compressible air bubbles interfere with the equal distribution of the applied pressure throughout the dough. If two bubbles are pictured as near to each other, the pressure on all sides can be assumed to be at a maximum, except on their adjacent sides, where it would be mutually reduced. The two bubbles about to coalesce in Figure 4(2) seem to offer graphic support of this theory. If the pressure on adjacent sides is thus reduced, the bubbles will move toward each other at an accelerating rate and coalesce.

Such differences in translucency as were observed were explainable in terms of bubble differences, and could not be related to yellow pigment, or amount of gray or red coloration. This indicates that light transmission readings alone are not suitable for evaluating the macaroni color of new durum hybrid selections as has been intimated in the literature.

Apparently no air was forced out of the macaroni discs used in these experiments. The air simply was coalesced into larger bubbles. Probably the same thing happens in the commercial macaroni process, although these experiments were not designed to test that point. It did seem that most macaroni products examined contained less air than these experimental discs. If this be true, it may be due to differences in mixers, or in mixing time. The small experimental mixer used here had small round pins which may incorporate more air into the dough than the flat paddle-shaped mixers used commercially.

However, the general implications of this study should be applicable to commercial processing. Translucency in macaroni products depends primarily upon pressure, both its intensity and its duration. Pressure produces translucency by coalescing the air bubbles. Small scale "slab tests" made without the application of pressure will necessarily give an opaque product.

Summary

Macaroni discs which had received no pressure contained great numbers of air bubbles less than 20 μ in diameter, and were very opaque. Application of 3,000 pounds pressure per square inch for four minutes coalesced these bubbles, giving translucent discs with bubbles 40 times as large in diameter, and reducing the number nearly 40,000 times. Light transmission increased six times. Intermediate treatments gave intermediate effects, but all results showed curvilinear relations.

Following application of pressure, there was a lag period before significant changes in bubble size or number were evident. This was interpreted as the "yield value" characteristic of plastic systems. At lower pressures the time factor was a very important influence. The first strands of macaroni pressed from a batch of dough are opaque because the pressure has not been operating for sufficient time. Laboratory "slab tests" which omit pressure will not give a translucent product.

Varietal differences in six widely different varieties were not striking. Monad and Kubanka gave somewhat larger bubbles and higher light transmission at the more severe treatments. Varietal differences

in light transmission could not be related to differences in yellow pigment, nor to undesirable red or gray colors.

Light transmission, a measure of translucency, is the resultant of two opposing and interdependent forces, decreasing bubble numbers and increasing bubble size. Because of its mathematical relation to size, bubble number changes faster than size; and therefore translucency increases as bubble number decreases, despite simultaneous increases in bubble size.

Pressure increases translucency by causing the bubbles to coalesce. A hypothesis is offered to explain why the bubbles coalesce.

Acknowledgment

The authors wish to acknowledge the assistance of J. Harwood Longwell in securing the data on bubble diameters.

Literature Cited

Alsberg, C. L.
1939 Durum wheats and their utilization. Wheat Studies of the Food Research Inst. **15** (7): 337–364.
Binnington, D. S., and Geddes, W. F.
1936 Experimental durum milling and macaroni making technique. Cereal Chem. **13**: 497–521.
Cunningham, R. L., and Anderson, J. A.
1943 Micro tests of alimentary pastes. I. Apparatus and method. Cereal Chem. **20**: 171–185.
1943a Micro tests of alimentary pastes. II. Effects of processing conditions on paste properties. Cereal Chem. **20**: 482–506.
Fifield, C. C.
1934 Experimental equipment for the manufacture of alimentary pastes. Cereal Chem. **11**: 330–334.
——, Smith, G. S., and Hayes, J. F.
1937 Quality in durum wheats and a method for testing small samples. Cereal Chem. **14**: 661–673.
Gortner, R. A.
1938 Outlines of Biochemistry. 1017 pp. John Wiley and Sons, New York.
Harris, R. H., and Sibbitt, L. D.
1942 Experimental durum milling and processing equipment, with further quality studies on North Dakota durum wheats. Cereal Chem. **19**: 388–402.

A MICRO-MILLING TECHNIQUE USING THE HOBART GRINDER[1]

KARL F. FINNEY and WILLIAM T. YAMAZAKI[2]

(Received for publication April 25, 1946)

Several workers including Johnson and Swanson (1942), McCluggage (1943), Lamb (1944), and Finney and Yamazaki (1946) have used a Hobart or some other type of grinder for obtaining flour for testing and evaluating the quality characteristics of small samples of commercially and experimentally grown wheat varieties. These flours have been comparatively high in ash content, however, due to relatively large quantities of finely divided bran particles. Considerable work with 100-g wheat samples has been carried out in the Federal Soft Wheat Laboratory in an effort to reduce the ash of flour obtained in this manner. This paper describes a micro-milling procedure involving two or three preliminary breaks through the rolls of the Tag-Heppenstall moisture meter and one break and two reductions on a Hobart grinder, and presents data showing the techniques responsible for obtaining flours having ash and protein contents comparable to those obtained by the usual experimental milling procedures.

Materials

Two wheat composites, one hard and the other soft red winter, were used in the preliminary work which established the amount and time of temper, Hobart grinder settings, number and size of sieves, sifting time, and other important techniques constituting the milling procedure. The hard winter composite consisted of seven varieties from each of four locations in the central and southern great plains. The soft winter composite consisted of 43 samples representing several varieties and locations in the soft winter wheat area.

The procedure described herein was applied to about 300 additional individual samples of soft red winter, white, hard red winter, and hard red spring wheat varieties from representative locations in the entire wheat-producing area of the United States, except the Pacific Coast.

Method

Previous to milling on the Hobart grinder, each cleaned 100-g wheat sample was run through the Tag-Heppenstall moisture meter

---

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associate Chemist and Junior Chemist, respectively, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

(first preliminary break). On the basis of the wheat moisture, each Tag-broken sample of hard winter and spring wheat was given a first temper to 14% moisture for 48 to 72 hours. Soft winter wheats were tempered to 12.5%. Limited data, however, have indicated that a first temper time of 18 to 24 hours was sufficient. A second temper of 2% was added to all samples 45 to 60 minutes before milling. Each sample was again run through the Tag-moisture meter one to three times (additional preliminary breaks) to shatter the kernels completely, after which it was subjected to one break and two reductions on the Hobart grinder with three siftings on a Ro-Tap. The relatively simple flow sheet showing Tyler sieves (meshes per inch) and grinder settings (Nos. 2, 1¼, and closed or 1 −) is shown in Figure 1.

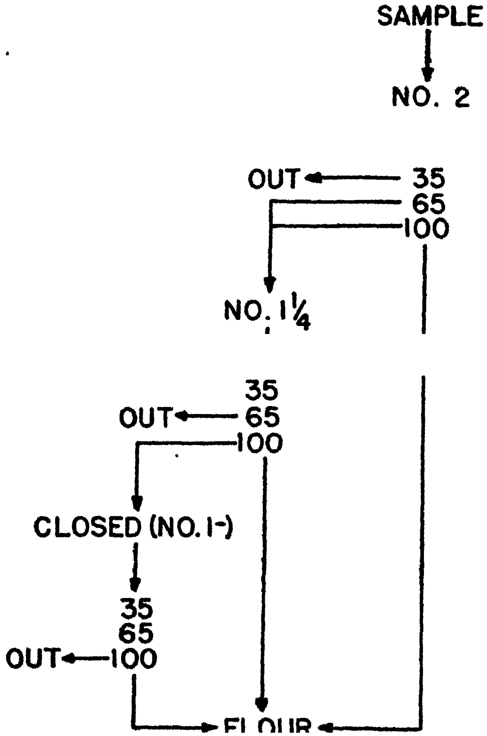


Fig. 1. Milling flow sheet for obtaining low-ash flours using a Hobart grinder.

It is extremely important that the wheat and middlings be fed slowly. For 100-g samples the wheat-feed time was about 45 to 60 seconds. The middlings were fed slowly so as not to overload the grinder as indicated by the sound of the motor and burrs. In each of the two reductions, the middlings were run through a second time to push through middlings which had lodged at any point. The sifting times for hard winters and springs were usually 1, 2, and 2 and for soft red winters and whites 2, 3, and 3 minutes for the first, second, and third siftings, respectively. In all instances, however, the bran was weighed at ½-minute intervals and was usually discarded after ½ to 1 minute. This was, as a rule, sufficient time to give a bran weight of 29 to 31 g. Bran weights appreciably greater than 31 g after 1 minute of sifting usually contained middlings, most of which passed through the sieve after an additional ½ to 1 minute of sifting.

During the second grinding (first reduction) if the overs of the 65-mesh sieve are run through first and followed by the overs of the 100-mesh sieve, a finer granulation will be obtained than if the overs of the two sieves are combined previous to reduction.

Another pertinent point in milling on the Hobart grinder is the proper adjustment of the burrs. The distances between the burrs for the 2, 1¼, and closed settings are such that when the grinder is running empty the burrs will not touch at a setting of 1⅜, will not touch or touch very faintly at 1¼, will just touch at several points over their surface at 1⅛, and will distinctly touch at many points at a setting of 1. The closed setting of 1 — should produce a slight howl or screeching sound. Other sets of burrs or other Hobart grinders may necessitate the use of different grinder settings in order to maintain the approximate weights of overs given in Table I. The average total time required for running a sample through the Tag, grinding, sifting, weighing, labeling, and mixing the products, calculating flour yield, and cleaning out the grinder for the next sample was 17 to 20 minutes.

The left hand pulverizing burrs of a model 1044 Hobart grinder were used for the milling work reported herein. As a result of co-operative work with the Hobart Manufacturing Company, the burrs (both rotor and stator) were standardized to an average depth of openings of 0.004 inches.

When a 2% second temper was used, *hard wheat* flours contained about 15% moisture. To insure safer storage, their moisture contents were reduced to about 14% by drying for 2 hours in a forced air oven held at room temperature after simply removing their ½ pint-container caps.

Experimental Results

The factors of tempering, wheat feed time, and amount of preliminary breaking or shattering previous to breaking the wheat in the Hobart grinder are extremely important in obtaining low ash flours. The data relative to these techniques are given in Table I and Figure 2.

The importance of the amount of temper is readily seen from the milling and analytical data for the hard winter composite shown in

TABLE I

MILLING AND CHEMICAL DATA FOR FLOURS MILLED ON THE HOBART GRINDER FROM 100-G SAMPLES OF A HARD RED WINTER WHEAT COMPOSITE TEMPERED TO DIFFERENT MOISTURE CONTENTS

Wheat moisture after 1st temper	Water added for 2nd temper	1st break feed time[1]	Overs 35 mesh sieve 1st sifting	Overs 65 mesh sieve		Overs 100 mesh sieve 3rd sifting	Flour		
				2nd sifting	3rd sifting		Yield	Moisture	Ash[2]
%	%	sec	g	g	g	g	g	%	%
11.0	0	58	24.7	3.26	0.79	1.97	65.8	11.5	.57
11.5	0	58	24.7	3.15	0.76	1.71	67.0	11.7	.55
12.0	0	57	25.1	3.42	0.84	1.82	67.4	12.0	.55
12.5	0	58	24.9	2.86	0.68	1.51	69.5	12.3	.54
13.0	0	58	25.5	3.07	0.78	1.49	68.7	12.7	.52
13.5	0	60	26.2	2.65	0.64	1.69	68.7	13.0	.50
14.0	0	59	26.3	2.77	0.63	1.52	69.0	13.3	.49
14.5	0	60	27.5	2.59	1.13	1.65	68.6	13.8	.48
13.0	2	60	28.9	2.88	0.70	1.41	66.4	13.6[3]	.47
13.5	2	60	29.7	2.84	0.81	2.26	66.3	13.6[3]	.45
14.0	2	60	30.3	2.74	1.13	2.26	66.2	13.9[3]	.45
14.5	2	64	31.2	2.92	1.31	2.44	65.4	13.9[3]	.45

[1] All samples received 3 preliminary breaks.

[2] 14% moisture basis.

[3] Dried for 2 hours in a forced air oven held at room temperature.

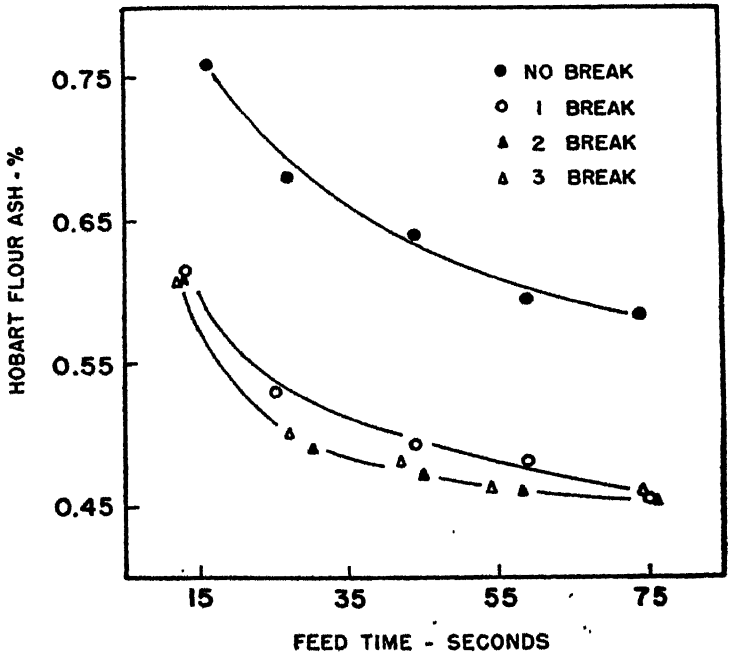


Fig. 2. Effect of preliminary breaking through the Tag-Heppenstall moisture meter and first-break feed time on the flour ash of 100-g wheat samples milled on the Hobart grinder.

Table I. As the moisture content of the grain was increased from 11% to 14.5% by varying the amounts of first temper water, the flour ash decreased from 0.57% to 0.48%. A second temper of 2% resulted in a further reduction in ash. For example, 2% second temper in combination with a first temper to 13 and 13.5% lowered the ash 0.05%. The second temper was somewhat less effective, however, when used in conjunction with a first temper to 14% and 14.5% moisture. The flour yields, which averaged about 68% when using only a first temper, were about 1.5 to 2% lower when a second temper also was used. The reduction in ash with the second temper, however, appears to more than compensate for the reduction in flour yields.

As the moisture added at the first temper was increased, the weight of overs on the 35-mesh sieve increased. The addition of 2% more water as a second temper resulted in additional and consistent increases. When using the 14.5 plus 2% second temper, however, there was a slight tendency for the flour to cake on the inside walls of the grinder. As a result, a first temper to 14% was used in conjunction with a 2% second temper in all routine work on hard wheats.

In a similar series for soft wheat, however, a first temper to more than 12.5% moisture with a 2% second temper resulted in caking on the walls of the grinder and a clogging of the sieves. Accordingly, a first temper to 12.5% with a 2% second temper was adopted for routine milling of typically soft wheat. More recent studies have indicated that semihard wheats should be first tempered to a moisture content of 13% to 14%; whereas, for the extremely soft samples, 12% moisture is preferable.

The weights of the overs of the various sieves for the 14% plus 2% second temper are typical, in general, of the results obtained with numerous samples of hard red winter as well as the other classes of wheat studied. Thus, these weights should be valuable as a guide to anyone interested in standardizing a Hobart grinder for milling small or possibly even large samples of wheat.

The amount of preliminary breaking between the Tag rolls previous to the first break through the Hobart grinder, and the first-break feed time is even more important than the amount of temper in reducing flour ash. The effect of these two factors on Hobart flour ash is strikingly illustrated in Figure 2.

All flours represented in Figure 2 were given a first temper to 14% and a 2% second temper. With no preliminary breaking through the rolls of the Tag moisture meter and with a first-break feed time of 16 seconds (time required for 100 g unbroken wheat to pass through grinder), a relatively high flour ash of 0.76% was obtained. Flour ash, however, decreased from 0.76% to 0.58% as the feed time was increased from 16 to 74 seconds.

Putting samples once through the Tag previous to the first break on the Hobart grinder and using a first-break feed time of 13 seconds lowered the flour ash from 0.76% to 0.62%, a reduction comparable to that obtained with no preliminary breaking and a long feed time of 59 seconds. Using one preliminary break, the flour ash was reduced from 0.62% to 0.45% with increasing feed time. Thus, at a given feed time, one break through the Tag rolls reduced the flour ash approximately 0.16%. The flour ashes resulting from the use of two and three Tag breaks when plotted against feed time define a single curve which is only slightly below that for one preliminary break, indicating that more than two breaks are unnecessary. Thus, it appears that for a normal hard wheat, such as is represented by the hard winter composite, two preliminary Tag breaks and a feed time of 1 minute will give a minimum ash content. It has been found, however, that for shriveled samples of wheat, as many as three or four Tag breaks were desirable. For example, Hobart flours having ash contents of 0.57%, 0.55%, 0.53%, and 0.52% were obtained from an appreciably shriveled, high-protein hard winter wheat as a result of the use of one, two, three, and four preliminary breaks, respectively. Thus, there is the probability of a lower ash to be gained and only a few seconds to be lost by passing the sample through the Tag rolls an extra time or two.

The Hobart milling procedure described herein has been applied to more than 300 samples (100-g) of soft red winter, white, hard red winter, and hard red spring wheats grown throughout the entire wheat-producing area of the United States, except the Pacific Coast. Typical milling and analytical data for representative samples of these different classes of wheat are shown in Table II.

Within the group of soft red winter and white wheat flours from Wooster, Ohio, and East Lansing, Michigan, the Hobart flour ashes varied from 0.43% to 0.53%, half of them being 0.49% or lower. Within the group of hard red winters, the flour ashes varied from 0.47% to 0.54%, being somewhat higher than for the soft wheat flours. The flours from the hard red spring wheats grown at Bozeman, Montana, however, were appreciably higher in ash than either the soft or hard winter flours; whereas, those from Langdon, North Dakota, averaged slightly lower in ash content than the soft wheat flours.

The protein contents of the Hobart flours were lower than those of the wheats by 0.5% to somewhat over 1%, and in this respect are comparable to Buhler and Allis straight grade flours. The flour yields

TABLE II

Chemical and Milling Data for Different Classes and Varieties of Wheat Grown at Several Locations and for Flours Milled from Them on the Hobart Grinder

Variety and location	Wheat		Flour			
	Ash[1] %	Protein[1] %	Moisture %	Ash[1] %	Protein[1] %	Yield g
SOFT RED WINTER AND WHITE						
Wooster, Ohio						
Clarkan	1.87	12.3	14.3	.49	11.1	65.5
Trumbull	1.80	11.5	14.3	.51	10.4	66.0
Fairfield	1.74	11.2	14.3	.50	9.8	65.3
Thorne	1.81	12.6	14.4	.48	11.4	64.5
Wabash	1.78	12.2	14.4	.43	11.0	65.4
Blackhawk	1.80	12.5	14.4	.46	11.5	68.3
Am. Banner	1.80	11.9	14.3	.53	10.9	66.4
E. Lansing, Mich.						
Clarkan	1.80	9.3	14.2	.52	8.5	64.7
Trumbull	1.71	9.0	14.1	.49	8.1	66.9
Fairfield	1.69	8.7	14.2	.53	7.5	64.6
Thorne	1.74	9.1	14.1	.53	8.3	64.8
Wabash	1.65	9.0	14.4	.47	8.2	66.2
Blackhawk	1.75	10.3	14.4	.49	9.0	67.5
Am. Banner	1.71	9.2	14.2	.52	8.2	66.9
Composite of several varieties and stations						
S.R.W. Std.	1.73	10.6	14.2	.45	9.5	65.7
HARD RED WINTER						
Central district composites						
Kharkof	1.88	14.8	13.9	.51	13.5	62.5
Blackhull	1.83	14.3	14.4	.52	13.4	65.9
Tenmarq	1.83	14.2	14.0	.50	13.0	64.6
Early B. H.	1.75	13.1	14.2	.49	12.4	66.4
Pawnee	1.62	14.3	14.7	.51	13.2	65.5
Comanche	1.73	14.5	14.7	.51	13.8	64.5
Chiefkan	1.75	13.8	14.2	.54	13.2	65.2
Wichita	1.72	14.1	14.3	.53	13.5	66.4
Woodward, Okla.						
Kharkof	1.51	9.8	14.8	.52	8.9	67.3
Blackhull	1.35	9.7	14.9	.51	8.5	67.3
Tenmarq	1.32	10.5	14.5	.53	9.6	68.1
Early B. H.	1.58	11.3	14.3	.47	10.6	65.4
Pawnee	1.38	10.3	14.5	.52	9.5	66.5
Comanche	1.47	11.0	14.6	.50	10.3	67.4
Chiefkan	1.45	10.5	14.6	.50	9.7	67.4
Wichita	1.48	10.9	14.5	.51	10.1	64.7
Composite of several varieties and stations						
H.R.W. Std.	1.45	13.6	14.5	.45	13.0	67.7

TABLE II (Continued)

Variety and location	Wheat		Flour			
	Ash [1] %	Protein [1] %	Moisture %	Ash [1] %	Protein [1] %	Yield g
			HARD RED SPRING			
Bozeman, Mont.						
Thatcher	1.56	11.8	14.7	.54	11.3	67.1
Pilot	1.66	11.5	14.7	.52	10.9	62.5
Mida	1.75	11.2	15.5	.55	10.7	64.4
Cadet	1.65	13.1	14.8	.59	12.8	63.6
Newthatch	1.65	12.3	15.2	.53	12.1	68.1
Marquis	1.68	12.9	14.9	.57	12.6	63.0
Ceres	1.70	11.8	14.8	.57	11.2	63.8
Langdon, N. D						
Thatcher	1.66	14.3	14.1	.51	13.6	63.3
Pilot	1.65	14.3	14.1	.46	13.2	60.5
Mida	1.55	14.4	14.2	.48	14.0	63.6
Cadet	1.54	14.4	14.2	.46	14.2	63.6
Newthatch	1.66	15.3	14.5	.49	15.2	66.4
Rival	1.58	14.6	14.7	.53	14.2	67.8
Regent	1.56	15.0	14.3	.46	14.7	65.9

[1] 14% moisture basis

averaged about 65 g from somewhat over 100 g of tempered grain and appear to be satisfactory in view of the small quantity of wheat milled.

The data presented in Table II show conclusively that a straight-grade flour with ash and protein levels comparable to those expected on the Buhler and Allis experimental mills can be obtained by milling 100-g wheat samples on the Hobart grinder. The ash contents of the Hobart-milled flours are comparable to those reported by Geddes and Aitken (1935) for flours obtained on their miniature, Allis-type experimental mill.

Summary

A comparatively simple milling procedure involving one or two preliminary breaks through the rolls of the Tag-Heppenstall moisture meter and one break and two reductions on the Hobart Coffee grinder is described whereby straight grade flours having ash and protein contents approaching those expected on Buhler and Allis experimental mills can be obtained from 100-g wheat samples.

Proper tempering, preliminary breaking or shattering of the wheat, and first-break feed time were about equally responsible for the reduction in flour ash content from somewhat under 1% to 0.45%.

Using the procedure and technique described, the Hobart grinder is useful as a simple and inexpensive micro-flour mill.

Acknowledgment

Credit is due the Hobart Manufacturing Company for its cooperation in work concerned with the effect of size of burr openings on flour yield and ash content.

Literature Cited

Finney, K. F., and Yamazaki, W. T.
1946 Water retention capacity as an index of the loaf volume potentialities and protein quality of hard red winter wheats. Cereal Chem. 23: 416–427.
Geddes, W. F., and Aitken, T. R.
1935 An experimental milling and baking technique requiring 100 grams wheat. Cereal Chem. 12: 696–707.
Johnson, J. A., Jr., and Swanson, C. O.
1942 The testing of wheat quality by recording dough mixer curves obtained from sifted wheat meals. Cereal Chem. 19: 216–229.
Lamb, C. A.
1944 Sifted wheat meal mixograms for selecting soft wheat varieties. Cereal Chem. 21: 57–64.
McCluggage, M. E.
1943 Micro milling and baking of small samples of wheat. Cereal Chem. 20: 185–193.

A STUDY OF SORGHUM AND SORGHUM STARCHES [1]

F. E. Horan [2] and Marie F. Heider

Midwest Research Institute, Kansas City, Missouri

(Received for publication May 23, 1946)

During the years of the war circumstances have made it extremely difficult for the manufacturers of starch, principally corn starch, to receive ample supplies of source materials. As a consequence, the wet-millers have been forced on a few occasions in recent years to resort to other starchy cereals, such as wheat and sorghum. New milling techniques have been developed by Slotter and Langford (1944) for the processing of wheat, owing to the unique doughing characteristics of the protein in this species of grain. The same procedures used with corn were applied to sorghum grains, but the results were not entirely satisfactory and undoubtedly many improvements will be added in connection with the processing of these latter grains.

Martin (1937) describes sorghum as a comparatively new crop in the United States, although it was cultivated as far back as 700 B.C. In the dry, hot, midwestern and southwestern states sorghum is greatly appreciated as a source of grain and forage for livestock. In general, sorghums are divided among several different groups that have distinct uses in this country. The grain sorghums, such as kafir, milo, hegari, feterita, and darso, are grown primarily for their grain. The sorgos (sweet sorghums or cane) are grown for the production of syrup or for forage.

---

[1] Presented before the Atlantic City meeting of the American Chemical Society, April 8–12, 1946.
[2] Corn Products Refining Company Research Fellow.

In the present discussion interest is focussed on the groups which might be important in supplying material for the starch industry, namely, sorgos and grain sorghums. The former have a disadvantage in that they grow tall and are therefore more difficult to harvest. But the recent universal acceptance of suitable new grain sorghum varieties and the use of the combine harvester-thresher will undoubtedly have a marked effect upon the agriculture of sorghum-producing areas. According to the Crop Reporting Board (1945) the acreage harvested for grain has increased from five million acres for the 1934–43 average to over six million for 1945. The average production of grain for the 1934–43 period was 70 million bushels, as compared with 96 million for 1945. Extensive work is being done at the Kansas, Nebraska, Oklahoma, and Texas Agricultural Experiment Stations by the plant breeders in an attempt to develop acceptable combine types. In view of the trend towards an increased production of sorghums and a greater demand for them by industry, the present work is concerned with collecting more information about sorghums and their starches.

Chemical Analyses of Sorghum Grains

To assist in the evaluation of the industrial potentialities of the many varieties of sorghums it is necessary to have proximate chemical analyses made on the grains. Some results on the standard varieties may be found in the literature, as reported by Baird (1910), Francis and Smith (1916), Edwards and Curtis (1943), MacMasters and Hilbert (1944), and Barham, *et al.* (1946), but due to the greatly expanded programs of the sorghum plant breeders in the past few years many new types have appeared. In all, 101 samples were analyzed, of which 14 were forage types and 87 grain sorghums. The method used for protein was the official A.O.A.C. (1940) Kjeldahl-Gunning method. The crude fat determinations were made by extracting the sorghum samples (ground to approximately 20-mesh) with Skellysolve "F-95" for six hours using a Butt extraction tube. The starch was determined by a polarimetric method used by Earle and Milner (1944) on corn samples.

The results of analyses for many of the newer varieties are summarized in Tables I and II. The results in Table I are arranged according to the four states from which samples were received. Table II contains more detailed information concerning the individual varieties examined. All of these grains were grown at the individual experiment stations, and for the most part represent different stages in the cross-breeding program. On an average basis, the values for the grain sorghums were somewhat higher in protein and starch and slightly lower in oil than the forage sorghums. Since the grains were not all produced in the same year nor in the same locality, the data are insufficient to characterize the grains according to variety. The majority of the grain sorghums from the four states had approximately the same proportions of protein, crude fat, and starch, a fact which is important in considering their general industrial utilization. Some exceptional values were obtained with a Texas sample which analyzed 7.7% protein, 2.5% crude fat, and 81.8% starch. A low crude fat content of 2.5% was obtained with one Nebraska sample, whereas 4.3% was obtained with another one. The unusually low starch content of 48.0%, which was undoubtedly due to the glumes adhering to the kernels, was found in still another Nebraska variety.

TABLE I

CHEMICAL ANALYSES OF SORGHUM GRAINS
(Moisture-free basis)

State	No. of samples	Sample type	Average protein[1]	Range in protein	Average crude fat	Range in crude fat	Average starch	Range in starch
			%	%	%	%	%	%
Kansas	6	Forage	12.8	12.0–13.4	4.1	3.5–4.4	69.9	61.3–73.5
	3	Nonwaxy	12.7	12.0–13.1	3.9	3.5–4.4	70.6	68.0–72.4
	3	Waxy	12.9	12.1–13.4	4.3	4.2–4.4	69.2	61.3–73.5
	22	Grain	13.6	11.9–15.4	3.7	2.9–4.1	71.7	68.1–74.8
	19	Nonwaxy	13.7	12.1–15.4	3.7	2.9–4.1	71.4	68.1–74.8
	3	Waxy	13.0	11.9–13.5	3.5	3.3–3.6	73.6	72.2–74.4
	28	Forage and grain, all samples	13.5	11.9–15.4	3.8	2.9–4.4	71.3	61.3–74.8
Nebraska	8	Forage	10.9	9.1–13.6	3.9	3.2–4.4	68.8	48.0–78.5
	7	Nonwaxy	11.0	9.1–13.6	3.9	3.2–4.4	68.9	48.0–78.5
	1	Waxy	10.7	—	4.3	—	67.6	—
	39	Grain	12.0	8.7–15.2	3.5	2.5–4.5	71.9	66.6–77.7
	35	Nonwaxy	11.9	8.7–15.2	3.4	2.5–4.3	72.1	66.6–77.7
	4	Waxy	13.4	12.0–14.7	4.0	3.4–4.5	70.2	67.7–73.4
	47	Forage and grain, all samples	11.8	8.7–15.2	3.6	2.5–4.5	71.4	48.0–78.5
Oklahoma	0	Forage	—	—	—	—	—	—
	5	Grain	13.1	11.9–15.6	3.5	3.0–4.1	69.3	62.8–74.3
	2	Nonwaxy	14.4	13.2–15.6	3.4	3.0–3.8	66.5	62.8–70.3
	3	Waxy	12.3	11.9–12.7	3.5	3.2–4.1	71.1	67.3–74.3
	5	Forage and grain, all samples	13.1	11.9–15.6	3.5	3.0–4.1	69.3	62.8–74.3
Texas	0	Forage	—	—	—	—	—	—
	21	Grain	11.1	7.7–14.3	3.3	2.5–4.2	73.9	67.8–81.8
	7	Nonwaxy	9.8	7.7–11.4	3.5	2.5–4.2	75.1	67.8–81.8
	14	Waxy	11.8	8.9–14.3	3.2	2.5–3.8	73.3	71.1–77.0
	21	Forage and grain, all samples	11.1	7.7–14.3	3.3	2.5–4.2	73.9	67.8–81.8

[1] Protein calculated as N × 6.25.

Waxy starch, which contains approximately 100% of the branched chain starch fraction (amylopectin) and is most easily recognized by the reddish-brown color it stains with iodine, has been found in certain varieties of sorghum. The nonwaxy starch, which stains blue with iodine due to presence of the linear starch fraction (amylose), occurs in the majority of sorghum varieties. In averaging the analytical results for the 87 samples of grain sorghums there appeared to be little difference in the proportions of protein, crude fat, and starch in samples which contained waxy and nonwaxy starches. The work of MacMasters and Hilbert (1944), however, indicated that there is a characteristic difference in the composition of waxy and nonwaxy types of sorghum, but their reported results were based on a limited number of samples.

Amylose Content of Sorghum Starches

Circumstances during the war created a demand for the waxy starches, principally as a replacement for tapioca starch, as reported by Hixon and Sprague (1942). The agronomists succeeded in developing

TABLE II

CHEMICAL ANALYSES OF SORGHUM GRAINS

Sample	Crop year	Moisture	(Moisture-free basis)		
			Protein	Crude fat	Starch
		%	%	%	%
Kansas:					
Forage, nonwaxy					
Atlas, C.I. 899	1944	9.7	12.0	4.4	72.4
Early Sumac, C.I. 1119	1944	10.7	13.0	3.5	68.0
Kansas Orange, C.I. 107	1944	10.4	13.1	4.0	71.6
Forage, waxy					
Waxy Atlas, H.C. 42-20	1944	9.6	12.1	4.3	73.5
Leoti Red, C.I. 1120	1944	10.6	13.4	4.4	61.3
Leoti x Atlas, H.C. 42-27	1944	9.6	13.2	4.2	72.8
Grain, nonwaxy					
Pink Kafir, C.I. 432	1944	9.7	12.6	3.7	71.0
Western Blackhull, C.I. 906	1944	9.8	13.6	3.7	71.9
Club Kafir, C.I. 901	1944	9.9	13.5	2.9	72.4
Finney Milo, C.I. 1089	1944	10.6	13.9	3.7	72.0
Feterita, C.I. 182-1	1944	9.7	15.4	3.5	70.2
Westland, G.C. 296	1944	9.6	13.4	3.8	68.9
Martin, S.A. 5330	1944	9.2	14.3	3.6	68.3
Early Kalo, C.I. 1009	1944	9.2	13.6	3.2	71.1
Midland, H.C. 617	1944	9.3	13.2	3.5	71.8
Wonder Club	1944	9.8	15.1	3.7	68.1
Dwf. Feterita x Dwf. Freed	1944	10.5	14.4	3.9	69.7
White Kalo Sel., 44-2112	1944	11.7	13.3	3.6	72.8
White Kalo Sel., 44-2117	1944	11.9	12.1	3.8	74.8
White Kalo Sel., 44-2119	1944	12.0	13.4	4.0	73.8
Leoti x (Club x Custer), 44-2115	1944	11.9	13.2	4.1	73.1
Waxy Hybrid, 44-2005	1944	12.1	13.2	3.8	73.4
Waxy Hybrid, 44-2021	1944	12.3	14.5	4.1	70.3
Waxy Hybrid, 44-2047	1944	12.2	13.7	3.6	70.9
Waxy Hybrid, 44-2049	1944	12.3	13.8	4.1	72.1
Grain, waxy					
Cody, H.C. 39-142	1944	10.2	11.9	3.3	74.4
Waxy Hybrid, 44-2020	1944	12.3	14.0	3.6	74.2
Waxy Hybrid, 44-2063	1944	12.0	13.5	3.6	72.2

TABLE II—(*Continued*)

Sample	Crop year	Moisture	(Moisture-free basis)		
			Protein	Crude fat	Starch
		%	%	%	%
Nebraska:					
Forage, nonwaxy					
Fremont, Nebr. 32	1944	9.0	12.9	4.0	67.0
Black Amber, F.D. 7038, Nebr. 19	1944	8.3	13.6	4.4	48.0
Kansas Orange, Nebr. 18	1944	9.7	9.6	4.2	71.4
Rox Orange, Nebr. 24	1944	9.9	10.0	3.8	71.3
S.A. 5023-19-4-3-1, N 176	1944	9.8	9.1	3.3	78.5
Norkan, C.I. 1116, Nebr. 38	1944	9.5	9.4	4.0	76.2
S.A. 5023-19-4-31, N 176	1945	9.0	12.1	3.2	70.4
Forage, waxy					
Leoti, C.I. 1120, Nebr. 26	1944	9.2	10.7	4.3	67.6
Grain, nonwaxy					
Caprock, Nebr. 58	1944	9.3	13.2	3.4	71.2
Western Blackhull, Nebr. 14	1944	9.4	9.7	3.3	75.7
Western Blackhull x Day, Nebr. 63	1944	9.9	12.1	3.5	73.3
Western Blackhull x Day, Nebr. 44	1944	9.3	9.6	4.1	72.9
Western Blackhull x Day, Nebr. 45	1944	9.5	13.4	3.6	72.7
Coes, Nebr. 28	1944	9.5	11.6	3.9	72.3
Club x Day No. 16, N 212	1944	9.5	9.3	3.1	74.8
Martin, Nebr. 59	1944	9.2	14.3	3.6	70.3
White Darso (Ks. 33-378), Nebr. 31	1944	9.6	11.2	4.3	69.5
Bonita, Nebr. 50	1943	9.4	13.4	3.0	71.3
Yellow Darso (813 x 615–36 x 12), N 134	1944	9.5	11.2	2.9	75.5
Chiltex, C.I. 874, Nebr. 27	1943	9.0	15.1	2.9	71.3
Club, C.I. 901, Nebr. 15	1944	9.3	12.6	2.8	75.0
Plainsman, Nebr. 57	1944	9.7	13.2	3.2	72.4
Highland, Nebr. 30	1944	9.5	11.4	4.0	72.1
Wd (871 x 792) x 71-3-4, N 194	1943	9.1	12.8	3.7	72.1
Alliance, Nebr. 33	1944	9.4	12.9	3.7	71.3
Sedan Red Kafir, N 25	1944	9.3	10.5	3.6	73.5
S.A. 7103-3-5-5, N 70	1944	9.0	10.1	3.4	74.9
S.A. 7103-8-6, N 71	1944	9.3	8.7	3.1	77.6
S.A. 7078-7-2-7-6, N 208	1044	9.1	10.4	3.3	76.6
S.A. 7103-3-6, N 69	1944	9.7	9.0	3.5	77.7
S.A. 7003-3-9-6, N 207	1944	8.8	10.7	3.4	71.6
S.A. 7097, N 68	1944	9.3	9.8	3.7	75.6
S.A. 5149-4-1, N 202	1944	9.1	11.8	2.5	72.8
S.A. 5252-1, N 205	1944	8.9	15.2	4.3	67.6
S.A. 5146-1, N 180	1944	9.0	11.1	3.1	71.5
S.A. 7009, N 170	1944	9.5	12.9	3.6	70.4
Schrock, Nebr. No. 27	1945	9.1	12.9	3.3	67.0
Yellow Darso, Nebr. N 134	1945	9.6	12.1	2.8	71.0
White Darso, Nebr. 31	1945	9.3	12.4	4.0	67.0
S.A. 7103-3-6, Nebr. 69	1945	8.4	11.0	3.5	69.2
S.A. 7103-8-6, Nebr. 71	1945	10.3	12.3	3.1	70.6
Martin, Nebr. 59	1945	8.7	14.8	3.6	66.6
Midland, Nebr. 47	1945	9.2	13.0	3.4	71.3
Grain, waxy					
Cody H.C. 39-140, N 193	1944	9.3	12.0	3.4	73.4
Waxy Kafir, S.A. 7113, Nebr. 48	1944	9.4	13.0	4.4	71.3
Waxy Milo, S.A. 5053, Nebr. 49	1943	9.0	14.7	3.8	67.7
Waxy Sooner, S.A. 5227-5, N 195	1943	8.9	14.0	4.5	68.6

TABLE II—(*Continued*)

Sample	Crop year	Moisture	(Moisture-free basis) Protein	Crude fat	Starch
		%	%	%	%
Oklahoma:					
Grain, nonwaxy					
Shallu, C.I. 185	1945	10.2	15.6	3.8	62.8
Bonar (Durra) x Dwf. Brown Kaferita	1945	10.4	13.2	3.0	70.3
Grain, waxy					
Cody (Perkins)	1945	11.9	12.3	3.4	74.3
White Schrock	1945	11.8	12.0	4.1	67.3
71 x Leoti	1945	12.2	12.7	3.2	71.8
Texas:					
Grain, nonwaxy					
Waxy Club x Bonita, S.A. 5590-4	1945	9.0	7.7	2.5	81.8
Bonar Durra x Texas Dbl. Dwf., S.A. 5345-8-1-1	1945	9.1	9.7	3.6	76.2
Bonar Durra x Texas Dbl. Dwf., S.A. 5345-8-1-3	1945	9.3	9.5	2.9	75.6
Dbl. Dwf. White Sooner, S.A. 5155-5	1945	9.5	11.4	3.5	74.5
Dbl. Dwf. Yellow Sooner, S.A. 5155-36	1945	10.2	1.9	3.5	76.1
Shallu, S.A. 5375	1945	10.3	10.0	4.2	73.9
Dbl. Dwf. Shallu, S.A. 5523-18-2	1945	9.1	10.6	4.2	67.8
Grain, waxy					
Waxy Kafir, S.A. 5178	1945	9.0	14.0	3.4	71.3
Waxy Whiteseed, S.A. 5107-5-2-1	1945	9.3	11.5	3.8	72.8
Waxy Club x Waxy Whiteseed, S.A. 5580-2	1945	9.8	12.7	3.1	71.2
Waxy Club x Waxy Whiteseed, S.A. 5581-11	1945	9.6	11.2	3.4	77.0
Waxy Club x Waxy Whiteseed, S.A. 5581-27	1945	9.5	11.3	3.6	72.3
Waxy Club x Waxy Whiteseed, S.A. 5581-28	1945	9.4	12.0	2.7	75.3
Waxy Club x Bonita, S.A. 5590-50-1	1945	9.4	10.5	2.9	74.8
Waxy Club x Bonita, S.A. 5590-43	1945	8.3	11.5	2.5	73.7
Waxy Club x Bonita, S.A. 5590-50-2	1945	9.4	9.7	2.9	74.4
Dwf. Blackhull Waxy Kafir, S.A. 5036-1-3-4-2-2	1945	9.3	14.3	3.5	71.9
Dbl. Dwf. Waxy Blackhull, S.A. 5547	1945	9.1	12.9	3.3	74.1
Dwf. Yellow Waxy Milo, S.A. 7080	1945	9.0	13.0	3.2	71.1
Dwf. Yellow Waxy Milo, S.A. 5404	1945	9.5	8.9	3.5	72.7
Dalhart x Dwf. Yel. Waxy Milo, S.A. 5502-3-3	1945	8.8	11.5	3.7	73.3

suitable varieties of sorghum and other cereals which contained starches with practically 100% amylopectin characteristics. There are many indications that these starches will continue in importance in the years to come. At the same time, a starch with a very high proportion of amylose might also have industrial uses, possibly in the production of fibers, as indicated by Whistler and Hilbert (1944). For that reason, a search was made among the sorghum samples studied here for high-amylose starches.

Three methods are generally available for the estimation of the relative amounts of amylose and amylopectin in starches. (1) The starch may be dispersed in water by autoclaving and then saturated with a polar organic substance, such as *n*-butanol, which causes the amylose to precipitate while the amylopectin remains in solution; this is Schoch's (1942) method. (2) The starch may be dissolved in potassium hydroxide, then neutralized and titrated with iodine solution by means of the electrometric method devised by Bates, French, and Rundle (1943). (3) The starch may be dissolved in sodium hydroxide, neutralized, and then diluted to give a final concentration of 0.002% starch. Iodine solution is added and light transmittance measurements are then made with a spectrophotometer at a wave length of 610 mμ, according to the procedure of Kerr and Trubell (1943). McCready and Hassid (1943) have proposed a similar method. All of these methods require that the starch be isolated from the plant material and be essentially free from noncarbohydrate matter.

In many instances, however, it is advantageous to be able to estimate the relative amounts of amylose and amylopectin without first isolating the starch. Such a rapid method has been developed by Kerr[3] and it consists principally in dispersing 2 g of the ground grain in calcium chloride solution (sp. gr. 1.30 at 20°C, pH of 2.5) and measuring the optical rotation of this solution in order to calculate the amount of starch present. An aliquot of this calcium chloride solution sufficient to contain 0.04 g of starch is then diluted with water so that a final starch concentration of 0.002% is obtained. Iodine solution is then added and the light transmittance is measured at 610 mμ. A standard curve, obtained by plotting the logarithm of the percentage transmittance against the percentage of amylose, was prepared by using an amylose and amylopectin which had been fractionated from a representative sample of sorghum starch.

When this method was applied to the sorghum samples containing nonwaxy starches, it was found that the percentage of amylose ranged from 27 to 45%. However, by first separating the starch from the grain, defatting it with methanol, and then titrating with iodine by the electrometric method of Bates, French, and Rundle (1943), the amylose contents ranged only from 25 to 31%. It is apparent then that the rapid method does not give absolute values for amylose content. Nevertheless, it is still useful in obtaining a relative evaluation of a large number of grain samples.[4] For example, most of the waxy samples analyzed between 0 and 5% amylose. On the other hand, a pea starch, which was known to contain a high proportion of amylose, gave a value of 90% by this method. It is, therefore, concluded that the nonwaxy sorghum samples studied here contained the normal amount of amylose, namely, from 25 to 30%, and that none had an exceptionally high percentage of amylose.

---

[3] This method was obtained from Dr. R. W. Kerr of the Corn Products Refining Company in a private communication.

[4] A method developed by Nielsen and Gleason (1945) for the determination of starch by measuring the light transmission of starch-iodine solutions might also be useful in obtaining relative values of amylose content.

Properties of Sorghum Starches

The sorghum grains were processed for starch by using a laboratory method proposed by MacMasters and Hilbert (1944). The grain was steeped in 0.3% sulfur dioxide solution for 48 hours at 45°C. A 100-mesh metal screen sieve was used in place of the No. 17 standard bolting silk to separate the starch from the germ, bran, and larger fragments.

In general, the waxy starches did not separate so easily from the protein layer as the nonwaxy starches. It was also observed that the starch from grains containing water-soluble pigments was usually colored.

The pasting characteristics of the sorghum starches were examined by means of the Brabender Amylograph as described by Anker and Geddes (1944) and Brown and Harrel (1944), Scott and Stormer viscosities, and light transmittance studies. Typical amylograph curves for sorghum and corn starches, both waxy and nonwaxy, were made, employing 30 g of starch and 450 ml of distilled water. The viscosities of several sorghum and corn starches were determined by using the Stormer and Scott viscosimeters. Two-hundred ml of a 2% starch suspension was heated to boiling over an open flame, and then kept for exactly 10 minutes in a bath of boiling water. In both methods the viscosimeter cup was maintained at 90°C. The 100-g weight was used with the Stormer viscosimeter, and the time in seconds was recorded for one complete revolution of the dial pointer. In the case of the Scott viscosimeter the time in seconds required for 50 ml of the paste to flow through the orifice of the cup was recorded.

The transition point, which is defined as the temperature at which the first perceptible increase in viscosity takes place (Cook and Axtmayer, 1937), occurred in the amylograph curves at about 30 minutes for waxy and 40 minutes for nonwaxy starches, i.e., at a lower temperature for the waxy than for the nonwaxy (Figure 1). It is also apparent that the maxima of the curves for the waxy types are much larger than those for the nonwaxy. Microscopic examination showed that most of the nonwaxy starch granules had disintegrated completely after the peak in the curve had been reached. However, in the case of the waxy starches there were a few small, blue-staining granules (nonwaxy) which appeared to be unaffected during the pasting process.

The viscosity results, along with the maxima obtained with the amylograph, are summarized in Table III. In general, the viscosities

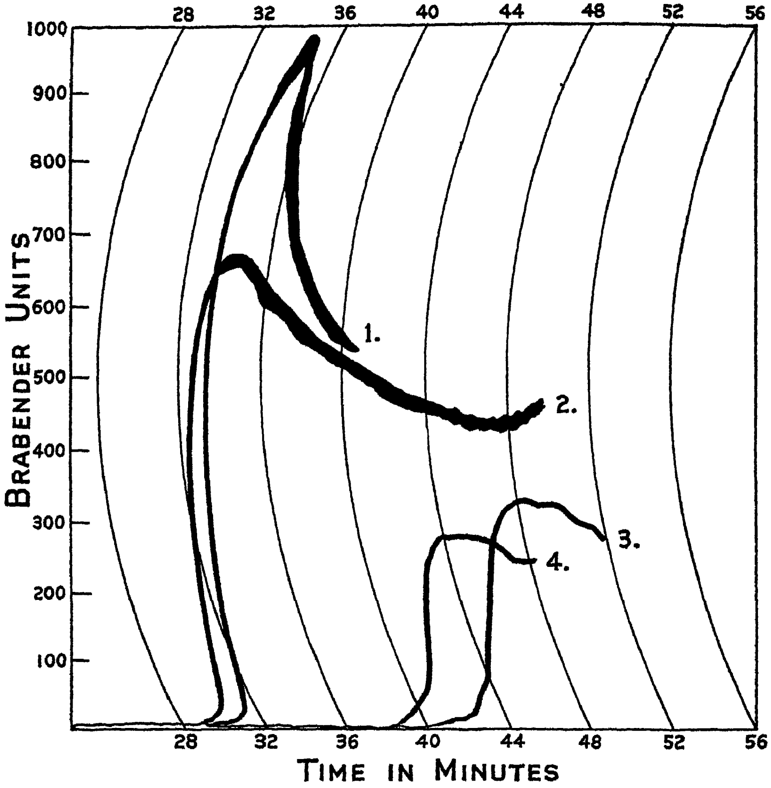


Fig. 1. Amylograph studies of starches (6.6% pastes).
1. Waxy sorghum (Cody H.C. 39–142)
2. Waxy maize
3. Nonwaxy sorghum (Atlas C.I. 899)
4. Nonwaxy corn

TABLE III
VISCOSITIES OF STARCHES

Sample	Stormer[1]	Scott	Brabender maxima
	Seconds	*Seconds*	
Corn starches			
Corn (commercial) (nonwaxy)	7	13	295
Waxy maize (commercial) (waxy)	9–10	17	680
Sorghum starches (nonwaxy)			
Western Blackhull, C.I. 906	8	14	400
Feterita, C.I. 182-1	8	14	365
Martin, S.A. 5330	8	13	400
Pink Kafir, C.I. 432	8	14	425
Atlas, C.I. 899	8	14	340
Sorghum starches (waxy)			
Leoti Red, C.I. 1120	15–12	37	900
Leoti x Atlas, H.C. 42-27	12–10	36	800
Cody, H.C. 39-142	16–12	58	990

---

[1] For the nonwaxy starches the Stormer values did not change with time. For the waxy starches, however, the time is recorded after the first and eleventh minute.

of the waxy starches were higher than those of the nonwaxy. The Stormer viscosities showed only small differences between corn and sorghum starches, either waxy or nonwaxy; the Scott viscosities differentiated more between the waxy corn and waxy sorghum; whereas the Brabender results indicated differences in both the waxy and nonwaxy types of corn and sorghum. The variations in the Brabender maxima for any one series of starches may be due to differences either in the grain variety or in the effects of processing. Somewhat similar viscosity results have been published by Barham and co-workers (1946).

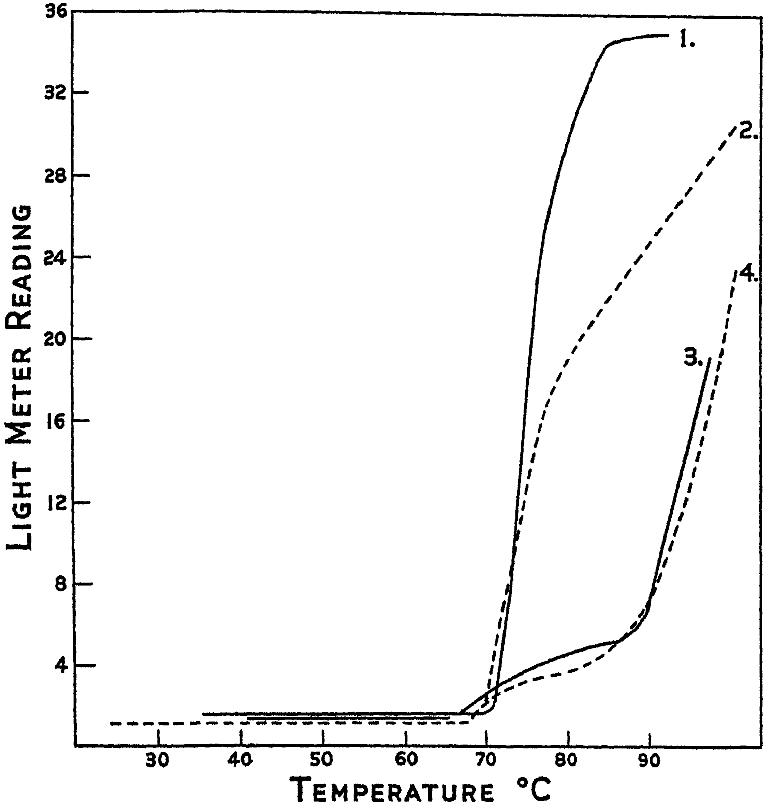


Fig. 2. Pasting of starches (0.51% pastes).
1. Waxy sorghum (Cody H.C. 39–142)
2. Waxy maize
3. Nonwaxy sorghum (Pink Kafir C.I. 432)
4. Nonwaxy corn

The method of Morgan (1940) was used in studying the pasting properties of several samples of starch. This method is based on the change in light transmission of starch slurries during heat gelatinization. Light from a 100-watt projection bulb was passed through a collimating lens on through the tube in which the paste was formed, and then onto the photoelectric cell. The heating was performed at the rate of about 2.5°C per minute. A Weston Illumination Meter, Model 603, was used to measure the intensity of the light. The values for the light transmitted, in arbitrary units, were plotted against the temperature. Representative results for corn and sorghum starches, given in Figure 2, point to the fact that there are no outstanding differences in the waxy starches of sorghum and corn; likewise, the nonwaxy starches of the same two cereal grains give quite similar light transmission curves. Photomicrographs of ungelatinized waxy and nonwaxy starch granules from corn and sorghum gave little or no evidence of any differences and contributed to the belief that the sorghum and corn starches have many properties in common.

Summary

Numerous samples of sorghum grains from Kansas, Nebraska, Oklahoma, and Texas were analyzed for protein, starch, and crude fat contents. The fact that the majority of the grain sorghums had approximately the same proportions of protein, starch, and crude fat may be important in considering their use in industry. In the line of exceptional varieties, one sample analyzed 7.7% protein, 2.5% crude fat, and 81.8% starch; another sample had a high crude fat content of 4.3%.

A rapid method involving the use of a saccharimeter and a spectrophotometer was employed for the estimation of the amylose contents of the sorghum starches. With the whole grain the results were high, but still of value in detecting any unusual properties with regard to amylose content. The nonwaxy sorghum samples contained the normal amount of amylose, namely, from 25 to 30% as determined by the electrometric iodine titration. No sample had an exceptionally high percentage of amylose.

Pasting characteristics of several waxy and nonwaxy sorghum starches, determined by means of the Brabender Amylograph, Scott and Stormer viscosities, and light transmission studies, showed a close similarity between corn and sorghum.

Acknowledgment

The authors wish to express appreciation to the Midwest Research Institute and to the Corn Products Refining Company for permitting the release of the data contained in this discussion. They also wish to thank the respective agricultural experiment stations for their complete cooperation in this work. Thanks are also due Dr. Ralph Kerr of Corn Products Refining Company for his suggestions concerning the determination of amylose in the whole grains

Literature Cited

Anker, C. A., and Geddes, W. F.
1944 Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21**: 335–360.
Association of Official Agricultural Chemists
1940 Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 5th ed., p. 26.
Baird, A. O.
1910 The chemistry of the kafir corn kernel. Okla. Agr. Expt. Sta. Bull. 89.
Barham, H. N., Wagoner, J. A., Campbell, C. L., and Harclerode, E. H.
1946 The chemical composition of some sorghum grains and the properties of their starches. Kans. State Coll. Tech. Bull. 61.
Bates, F. L., French, D., and Rundle, R. E.
1943 Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65**: 142–148.
Brown, R. O., and Harrel, C. G.
1944 The use of the amylograph in the cereal laboratory. Cereal Chem. **21**: 360–369.
Cook, D. H., and Axtmayer, J. H.
1937 Determining the gelatinization temperature of starches. Ind. Eng. Chem. (Anal. Ed.) **9**: 226–228.
Crop Reporting Board
1945 Bureau of Agricultural Economics, U. S. Dept. Agr., September 10.
Earle, F. R., and Milner, R. T.
1944 Improvements in the determination of starch in corn and wheat. Cereal Chem. **21**: 567–575.
Edwards, W. M., and Curtis, J. J.
1943 Grain sorghums, their products and uses. U. S. Dept. Agr., Bureau of Agr. and Ind. Chem. ACE-193, NM-229.
Francis, C. K., and Smith, O. C.
1916 The starches of the grain sorghums. Okla. Expt. Sta. Bull. No. 110.
Hixon, R. M., and Sprague, G. F.
1942 Waxy starch of maize and other cereals. Ind. Eng. Chem. **34**: 959–962.
Kerr, R. W., and Trubell, O. R.
1943 Spectrophotometric analyses of starches. Paper Trade Journal, Oct. 7.
MacMasters, M. M., and Hilbert, G. E.
1944 Glutinous corn and sorghum starches. Ind. Eng. Chem. **36**: 958–963.
Martin, J. H.
1937 Sorghum improvement. U. S. Dept. Agr. Yearbook Separate No. 1575, 523–560.
McCready, R. M., and Hassid, W. Z.
1943 The separation and quantitative estimation of amylose and amylopectin in potato starch. J. Am. Chem. Soc. **65**: 1154–1157.
Morgan, W. L.
1940 Pasting and identification of starches. Ind. Eng. Chem. (Anal. Ed.) **12**: 313–317.
Nielsen, J. P., and Gleason, P. C.
1945 Rapid determination of starch. Ind. Eng. Chem. (Anal. Ed.) **17**: 131–134.
Schoch, T. J.
1942 Fractionation of starch by selective precipitation with butanol. J. Am. Chem. Soc. **64**: 2957–2961.
Slotter, R. L., and Langford, C. T.
1944 Wheat starch manufacture. Ind. Eng. Chem. **36**: 404–408.
Whistler, R. L., and Hilbert, G. E.
1944 Mechanical properties of films from amylose, amylopectin, and whole starch acetates. Ind. Eng. Chem. **36**: 796–798.

COMPOSITION OF THE COMPONENT PARTS OF THE CORN KERNEL

F. R. EARLE, J. J. CURTIS, and J. E. HUBBARD

Northern Regional Research Laboratory,[1] Peoria, Illinois

(Received for publication May 10, 1946; presented at the Annual Meeting, May 1946)

The breeding of corn for commercial production has been guided almost entirely by agronomic factors such as yield, general appearance of the plant and grain, and resistance to disease and lodging. It is generally agreed that it would be desirable to have a high-protein corn for feeding and a high-oil corn for milling, but these factors have been given little consideration by the corn producers. Unless the milling industry can offer special inducements, production of high-oil corn is unlikely unless the agronomic characteristics are at least as favorable as those of other available varieties. There are significant differences in the oil contents of the hybrid corn varieties which are widely grown at the present time. A study of some of these varieties shows the possibilities of advantage in processing high-oil corn.

It should be emphasized that the data in this paper indicate that better hybrids can be developed for processing but are not to be considered as recommendation or condemnation of specific varieties.

If the high-oil corn is to be of special value to industry, it is essential that the increase in oil be largely in the germ. In order to show the location of the oil in current varieties of corn, 11 samples have been carefully separated by hand into four portions consisting of endosperm, germ, bran, and tip-cap. The samples were selected to include one yellow and one white hybrid variety of high-oil content and one each of low-oil content. Three other yellow hybrid varieties of intermediate oil content were included, one of them being represented by two samples grown at different stations in 1945 and by one sample grown at one of the same stations in 1942. A flint and a flour corn were also included to see if such diverse types would have unusual proportions.

Methods

The method of separation was similar to that of Hopkins, Smith, and East (1903) except that cold water was used to soak the corn to loosen the bran and the time of soaking was kept to the minimum necessary to permit the hand separation, usually between 5 and 30 minutes. In order to get an indication of the uniformity of the samples, each sample was divided into four portions of about 75 g and each portion separated into endosperm, germ, bran, and tip-cap fractions. After air-drying, the fractions were examined for completeness of separation and weighed. The physical proportions determined on the four replications were in such good agreement that the replicates were combined and only the average result is reported.

---

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

In preparing the samples for analysis, the endosperm was ground in a hammer mill, the bran and tip-cap in an intermediate model Wiley mill, and the germ was coarsely ground in a small attrition mill. Since the samples were air-dried before grinding it is considered that there would be no great change in moisture during grinding. The tip-cap fraction was so small that it was necessary to use composites for analysis. One composite represented six of the yellow double-cross hybrids and the other represented the flint corn, the flour corn, and the two white single-cross hybrid strains. These two composites had such similar analyses that the average of the two was used in connection with each of the 10 samples of grain. The tip-cap from the remaining sample was obtained later and the amount was so small that only a partial analysis could be made.

Samples of the grain and the fractions resulting from the separation were analyzed for moisture, ash, nitrogen, oil, sugar, and starch. The methods of the Association of Official Agricultural Chemists (1940) were used to determine moisture, ash, nitrogen, and sugar. Oil was determined as in the A.O.A.C. method for oil in cottonseed except that the sample was unwrapped after about 4 hours, ground in a mortar, and then extracted overnight. Starch was determined by the Clendenning polarimetric method (1945) using uranyl acetate as the protein precipitant.

Discussion

All analyses are reported on the moisture-free basis.

The identity of the corn samples and the chemical composition are shown in Table I. The physical and chemical composition of the fractions is shown in Table II. In Table III, derived from Table II by calculation, is shown the amount of each determined constituent in each fraction expressed as the percentage of the whole kernels. Also in Table III is shown the amount of each constituent which occurs in each fraction expressed as the percentage of the total amount of that constituent in the whole kernels.

The composition of the whole corn kernel was calculated from the composition of its parts, as given in the first part of Table III, by totaling for each constituent the percentage found in the four fractions of each sample of whole kernels. This calculated composition has been included in Table I for comparison with the composition obtained directly by analysis of the kernels. In general, the agreement is excellent, the principal disagreement being in the figures for starch in the flint and flour corns. Some of the differences in the ash and sugar results can be explained by the extraction of solubles from the whole grain during the soaking period. The amount of solubles was not measured for these samples, but in some later work it was found to be not more than 0.25% of the total sample. Of this amount approximately one-fourth was ash and one-fourth protein, and the remainder was assumed to be carbohydrate. Unless there is a special interest in the sugar or protein content of the bran, it is believed that the errors due to the extraction of soluble materials are insignificant.

TABLE I

COMPOSITION OF WHOLE CORN

Sample No.	Variety	Source	Crop year	Wt. per 1,000 kernels	Moisture-free basis					
						Ash	Protein (N × 6.25)	Oil	Sugar	Starch
				g		%	%	%	%	%
1	Iowa 939	Ames, Ia.	1942	305	Determined Calculated[1]	1.52 1.64	8.2 8.3	3.9 4.1	1.77 1.80	74.0 74.2
2	U. S. 13	Ames, Ia.	1942	284	Determined Calculated	1.47 1.45	8.1 7.8	4.3 4.2	1.79 1.56	73.7 74.0
3	U. S. 13	Ames, Ia.	1945	257	Determined Calculated	1.33 1.33	9.8 9.6	4.3 4.3	1.61 1.60	73.7 73.5
4	U. S. 13	Wooster, O.	1945	260	Determined Calculated	1.47 1.44	11.1 10.9	4.7 4.7	1.74 1.81	72.0 73.0
5	Illinois 201	Ames, Ia.	1942	299	Determined Calculated	1.58 1.67	8.4 8.3	4.7 5.0	2.22 1.71	73.1 73.6
6	U. S. 44	Ames, Ia.	1942	273	Determined Calculated	1.39 1.50	8.2 8.1	4.7 4.8	2.06 2.03	74.2 74.4
7	Illinois 200[2]	Illinois	1942	315	Determined Calculated	1.48 1.50	11.5 11.7	5.6 5.8	2.20 2.07	67.8 68.7
8	Ind. 33-16 × K64	Chillicothe, O.	1944	305	Determined Calculated	1.27 1.38	9.6 9.4	4.1 4.2	2.12 1.92	73.4 73.9
9	T18 × Tx4R3	Chillicothe, O.	1944	418	Determined Calculated	1.35 1.44	11.4 11.4	5.8 5.9	2.01 1.89	70.0 70.6
10	Gehu Flint	Bismarck, N. Dak.	1940	215	Determined Calculated	1.51 1.55	13.6 13.4	5.6 5.5	2.03 1.97	67.8 69.8
11	Mandan White Flour	Bismarck, N. Dak.	1940	225	Determined Calculated	1.49 1.68	13.2 13.3	5.6 5.7	2.14 2.07	66.8 69.1
	Average				Determined	1.44	10.3	4.8	1.97	71.5
	High				Determined	1.58	13.6	5.8	2.22	74.2
	Low				Determined	1.27	8.1	3.9	1.61	66.8

[1] Calculated from data in Tables II and III.
[2] Composite of seven samples.

The results of this study of hybrid corn are in excellent agreement with the more extensive study made in 1903 by Hopkins, Smith, and

TABLE II

COMPOSITION OF CORN FRACTIONS
(Moisture-free Basis)

Sample No.	Variety	Proportion of whole grain	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
		%	%	%	%	%	%
	Endosperm Portion						
1	Iowa 939	83.0	0.46	7.2	0.8	0.77	87.8
2	U. S. 13	82.7	.32	6.7	.7	.47	88.0
3	U. S. 13	83.5	.23	8.8	.8	.48	86.9
4	U. S. 13	82.7	.22	10.4	.9	.82	86.4
5	Illinois 201	82.1	.46	7.2	1.0	.48	87.8
6	U. S. 44	81.8	.27	6.9	.8	.70	88.9
7	Illinois 200[1]	80.3	.28	11.1	.9	.64	83.9
8	Ind 33-16 × K64	83.0	.32	8.6	.8	.64	87.2
9	T18 × Tx4R3	81.7	.23	10.8	1.1	.49	84.5
10	Gehu Flint	80.6	.24	12.8	.8	.73	84.7
11	Mandan White Flour	79.7	.33	12.8	.8	.81	84.7
	Average	81.9	.31	9.4	.8	.64	86.4
	High	83.5	.46	12.8	1.1	.82	88.9
	Low	79.7	.22	6.7	.7	.47	83.9
	Germ or Embryo Portion						
1	Iowa 939	10.5	11.31	18.9	31.1	10.36	9.0
2	U. S. 13	11.0	10.22	18.0	32.6	10.36	9.2
3	U. S. 13	10.2	10.50	19.0	34.9	11.46	5.1
4	U. S. 13	10.9	11.04	18.4	35.1	10.13	8.8
5	Illinois 201	11.9	10.31	17.3	34.0	10.80	8.1
6	U. S. 44	12.3	10.15	18.3	33.1	10.00	8.0
7	Illinois 200[1]	12.8	9.67	20.0	38.9	11.95	6.2
8	Ind 33-16 × K64	10.9	9.87	18.7	31.6	12.51	10.0
9	T18 × Tx4R3	13.1	9.38	18.2	37.9	11.51	10.0
10	Gehu Flint	13.5	9.50	20.2	35.3	10.00	8.0
11	Mandan White Flour	14.1	9.16	19.9	35.0	9.87	8.0
	Average	11.9	10.10	18.8	34.5	10.81	8.2
	High	14.1	11.31	20.2	38.9	12.51	10.0
	Low	10.2	9.16	17.3	31.1	9.87	5.1
	Bran Portion						
1	Iowa 939	5.7	1.01	3.8	1.2	.52	7.0
2	U. S. 13	5.5	.97	3.3	.7	.27	3.5
3	U. S. 13	5.2	.90	3.9	.9	.34	6.5
4	U. S. 13	5.4	.86	3.8	1.1	.36	8.2
5	Illinois 201	5.3	.97	3.8	1.2	.39	10.4
6	U. S. 44	5.1	.36	3.0	1.1	.29	7.7
7	Illinois 200[1]	6.2	.36	3.8	.7	.28	8.0
8	Ind 33-16 × K64	5.1	.36	2.9	.8	.19	7.0
9	T18 × Tx4R3	4.4	.29	3.6	1.1	.42	7.5
10	Gehu Flint	5.1	1.18	4.6	1.0	.32	7.0
11	Mandan White Flour	5.5	2.00	4.6	.9	.32	7.1
	Average	5.3	.84	3.7	1.0	.34	7.3
	High	6.2	2.00	4.6	1.2	.52	10.4
	Low	4.4	.29	2.9	.7	.19	3.5
	Tip Cap Portion						
3	U. S. 13	1.1	1.95	10.7	3.9		
	Composite[2]	.8 (Av.)	1.59	9.1	3.8	1.61	5.3
	Composite[3]	.8 (Av.)	1.43	9.2	3.7	1.44	5.3

---

[1] Composite of seven samples.
[2] Composite of six yellow dent varieties.
[3] Composite of one flint, one flour, and two white dent varieties.

TABLE III
Distribution of the Chemical Constituents in Corn
(Data Derived from Tables I and II)

Sample No.	Variety	Proportion of whole grain existing as indicated constituent in specified fraction					Proportion of total of indicated constituent existing in specified fraction				
		Ash	Protein (N ×6.25)	Oil	Sugar	Starch	Ash	Protein (N ×6.25)	Oil	Sugar	Starch
		%	%	%	%	%	%	%	%	%	%
	Endosperm Portion										
1	Iowa 939	0.38	6.0	0.7	0.64	72.9	23.3	72.6	16.6	36.1	98.1
2	U. S. 13	.26	5.5	.6	.39	72.8	18.2	71.3	13.3	25.0	98.3
3	U. S. 13	.19	7.3	.6	.40	72.6	14.4	76.4	14.6	25.0	98.7
4	U. S. 13	.18	8.6	.8	.68	71.4	12.6	78.9	16.6	37.3	98.0
5	Illinois 201	.38	6.0	.9	.39	72.1	22.7	72.0	17.4	23.0	97.9
6	U. S. 44	.22	5.6	.6	.57	72.7	14.7	69.5	13.3	28.2	97.8
7	Illinois 200 [2]	.22	8.9	.7	.51	67.4	15.0	75.7	12.8	24.8	98.1
8	Ind 33–16 × K64	.27	7.2	.7	.53	72.4	19.4	75.8	16.2	27.7	97.9
9	T18 × Tx4R3	.19	8.8	.9	.40	69.0	13.0	77.1	15.1	21.2	97.8
10	Gehu Flint	.19	10.3	.6	.59	68.3	12.5	77.3	11.5	29.9	97.9
11	Mandan White Flour	.26	10.2	.7	.65	67.5	15.7	76.6	11.9	31.2	97.7
	Average	25	7.7	.7	.52	70.8	16.5	74.8	14.5	28.2	98.0
	High	.38	10.3	.9	.68	72.9	23.3	78.9	17.4	37.3	98.7
	Low	.18	5.5	.6	.39	67.4	12.5	69.5	11.5	21.2	97.7
	Germ or Embryo Portion										
1	Iowa 939	1.19	2.0	3.3	1.09	0.9	72.4	23.9	81.0	61.5	1.3
2	U. S. 13	1.12	2.0	3.6	1.14	1.0	77.3	25.5	85.0	73.3	1.3
3	U. S. 13	1.07	1.9	3.6	1.17	.5	80.5	20.3	83.3	72.8	.7
4	U. S. 13	1.20	2.0	3.8	1.10	1.0	83.2	18.4	81.4	60.8	1.3
5	Illinois 201	1.23	2.1	4.0	1.28	1.0	73.6	24.9	80.9	75.1	1.3
6	U. S. 44	1.25	2.2	4.1	1.43	1.2	83.3	27.8	84.9	70.5	1.6
7	Illinois 200 [2]	1.24	2.6	5.0	1.53	.8	82.8	21.8	86.0	73.8	1.1
8	Ind 33–16 × K64	1.08	2.0	3.4	1.36	1.1	78.2	21.6	81.9	71.0	1.5
9	T18 × Tx4R3	1.23	2.4	5.0	1.46	1.2	85.2	20.9	83.6	77.2	1.7
10	Gehu Flint	1.28	2.7	4.8	1.35	1.1	82.9	20.4	87.1	68.7	1.5
11	Mandan White Flour	1.29	2.8	4.9	1.39	1.1	77.1	21.1	86.8	67.4	1.6
	Average	1.20	2.3	4.1	1.30	1.0	79.7	22.4	83.7	70.2	1.4
	High	1.29	2.8	5.0	1.53	1.2	85.2	27.8	87.1	77.2	1.7
	Low	1.07	1.9	3.3	1.09	.5	72.4	18.4	80.9	60.8	.7

---

[1] * = less than 0.05%.

[2] Composite of seven samples.

TABLE III—*Continued*

Sample No.	Variety	Proportion of whole grain existing as indicated constituent in specified fraction					Proportion of total of indicated constituent existing in specified fraction				
		Ash	Protein (N×6.25)	Oil	Sugar	Starch	Ash	Protein (N×6.25)	Oil	Sugar	Starch
		%	%	%	%	%	%	%	%	%	%
	Bran Portion										
1	Iowa 939	0.05	0.2	0.1	0.03	0.4	3.5	2.6	1.7	1.7	0.5
2	U. S. 13	.05	.2	*[1]	.02	.2	3.6	2.3	1.0	1.0	.3
3	U. S. 13	.05	.2	*	.02	.3	3.5	2.1	1.1	1.1	.5
4	U. S. 13	.05	.2	.1	.02	.4	3.2	1.8	1.3	1.1	.6
5	Illinois 201	.05	.2	.1	.02	.6	3.1	2.4	1.2	1.2	.7
6	U. S. 44	.02	.2	.1	.02	.4	1.2	1.8	1.2	.7	.5
7	Illinois 200[2]	.02	.2	*	.02	.5	1.5	2.0	.8	.8	.7
8	Ind 33–16 × K64	.02	.2	*	.01	.4	1.3	1.6	1.0	.5	.5
9	T18 × Tx4R3	.01	.2	*	.02	.3	.9	1.4	.8	1.0	.4
10	Gehu Flint	.06	.2	*	.02	.4	3.9	1.7	.9	.8	.5
11	Mandan White Flour	.11	.2	.1	.02	.4	6.6	1.9	.9	.8	.6
	Average	.05	.2	.1	.02	.4	2.9	2.0	1.1	1.0	.5
	High	.11	.2	.1	.03	.6	6.6	2.6	1.7	1.7	.7
	Low	.01	.2	*	.01	.2	.9	1.4	.8	.5	.3
	Tip Cap Portion										
1	Iowa 939	0.01	0.1	*	0.01	*	0.7	0.9	0.8	0.7	0.1
2	U. S. 13	.01	.1	*	.01	*	.8	.9	.7	.8	.1
3	U. S. 13	.02	.1	*	.02	0.1	1.6	1.2	1.0	1.1	.1
4	U. S. 13	.02	.1	*	.02	.1	1.0	.8	.8	.8	.1
5	Illinois 201	.01	.1	*	.01	*	.7	.7	.5	.6	.1
6	U. S. 44	.01	.1	*	.01	*	.8	.9	.6	.6	.1
7	Illinois 200[2]	.01	.1	*	.01	*	.7	.5	.4	.5	.1
8	Ind 33–16 × K64	.02	.1	*	.02	.1	1.1	1.0	.9	.8	.1
9	T18 × Tx4R3	.01	.1	*	.01	*	.8	.6	.5	.6	.1
10	Gehu Flint	.01	.1	*	.01	*	.8	.5	.6	.6	.1
11	Mandan White Flour	.01	.1	*	.01	*	.7	.4	.5	.5	.1
	Average	.01	.1	*	.01	*	.9	.8	.7	.7	.1
	High	.02	.1	*	.02	.1	1.6	1.2	1.0	1.1	.1
	Low	.01	.1	*	.01	*	.6	.5	.4	.5	.1

[1] * = less than 0.05%.

[2] Composite of seven samples.

East on their strains of corn selected for high and low protein and for high and low oil. This probably means only that hybridization has not changed the physical proportions of the kernel and that any conclusions to be drawn from the early data will apply equally well to present-day hybrids.

The utilization of the data of this study will depend entirely on the interest of the user. The primary purpose of this paper is to show the distribution of oil in the corn kernel and only relevant data will be discussed. For other purposes many interesting relationships might be found.

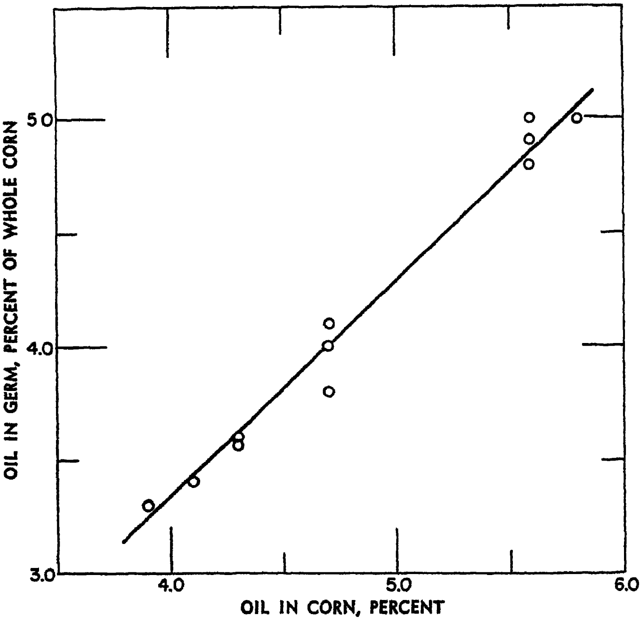


Fig. 1. Relation of oil in germ and oil in whole corn.

Figure 1 shows that proportion of the whole grain which occurs as oil in the germ plotted against the total oil in the sample. The correlation between the protein in the endosperm and that in the whole grain is equally good. Use of Figure 1 permits an estimate of the amount of oil which might possibly be recovered in processing a sample of corn of known total oil content. In illustration of this use, Table IV shows the possible recovery of oil from high-oil and low-oil corn. The calculations are based on 100 pounds of moisture-free corn and it is assumed that 100% of the germ will be separated free of other material and that the germ cake will contain 5% oil. These figures can be modified for any particular installation where the percentage of germ recovery and

TABLE IV
OIL YIELD FROM CORN
(Based on 100 pounds of moisture-free corn)

	Ill 200	Iowa 939
	Pounds	*Pounds*
Weight of oil in kernels	5.6	3.9
Weight of germ	12.8	10.5
Weight of oil in germ	5.0	3.3
Weight of oil in germ cake (5% residual oil)	.4	.4
Weight of oil recovered	4.6	2.9

the amount of nongerm material in the germ cake are known. Incomplete recovery of germ would reduce the oil yield proportionately. The presence of nongerm materials and excess oil in the germ cake would reduce the yield by equal amounts in the processing of high- and low-oil corn but the percentage reduction would be greater for the low-oil corn.

Summary

Eleven samples of corn grain were separated by hand into fractions representing the endosperm, germ, bran, and tip-cap. The grains and the resulting fractions were analyzed for moisture, ash, nitrogen, oil, sugar, and starch. The endosperm contains approximately 16% of the total ash, 75% of the total protein, 15% of the oil, 28% of the sugar, and 98% of the starch. The germ contains approximately 80% of the total ash, 22% of the protein, 84% of the oil, and 70% of the sugar. The data show high correlation between the amount of oil in the grain and the amount in the germ.

Acknowledgment

Seed samples were supplied by the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Iowa, Illinois, and Ohio experiment stations.

Literature Cited

Association of Official Agricultural Chemists
1940 The official and tentative methods of analysis (5th ed.).
Clendenning, K. A.
1945 Polarimetric determination of starch in cereal products. IV. Critical studies of methods for the determination of starch in whole wheat, granular, and patent flours. Can. J. Research B23: 239-259.
Hopkins, C. G., Smith, L. H., and East, E. M.
1903 The structure of the corn kernel and the composition of its different parts. Ill. Agr. Expt. Sta. Bull. 87.

INFLUENCE OF INGREDIENTS ON THIAMINE RETENTION AND QUALITY IN BAKING POWDER BISCUITS

ALICE M. BRIANT and MARCIA R. HUTCHINS

New York State College of Home Economics and Agricultural Experiment Station, Cornell University, Ithaca, New York

(Received for publication June 14, 1946)

In self-rising flour biscuits, Barackman (1942) reported 85% of the thiamine was retained if the pH of the baked product was 7.1 or lower. He obtained slightly better retention of thiamine when fluid milk was substituted for water. Williams and Fieger (1944) reported that thiamine content of enriched-flour biscuits decreased with increase in the pH of the dough mixture.

Because little is known of the retention of thiamine in home-baked enriched-flour products, a study was made of the effect of certain ingredients on thiamine retention in baking powder biscuits made by a household method. The effect of the variables on the quality of the biscuits was also studied.

Materials and Methods

A basic formula (Table I) was used for the biscuits, and variations from this were made in liquid and in leavening agent (Table II). A standard household method of preparing the biscuit dough was used.

TABLE I

BASIC FORMULA FOR BISCUITS

Ingredient	Weight
Flour, pastry	330 g[1]
Salt	6.0 g
Fat, hydrogenated vegetable	75.0 g
Baking powder, sodium aluminum sulfate-phosphate	12.3 g
Fluid whole milk	240 ml

[1] From 3 to 6 g additional flour was used in kneading and rolling.

The dough was rolled to ⅜-inch thickness, and the biscuits were cut two inches in diameter. They were spaced uniformly on a baking sheet and baked in a household type gas oven at 450°F for 12 minutes, or for 15 minutes when water was the liquid.

In Table II, the experiments are grouped according to the ingredients studied. For each day's baking, one batch for each variable in the group was prepared. In all, five batches for each variable were baked. A different bag of flour was used for each group of experiments, but all the flour was from the same mill lot.

Sampling methods were designed to reduce to a minimum the variation due to manipulation and to lack of homogeneity of the dough. The position of the biscuits relative to each other was maintained throughout the cutting, baking, and cooling processes. The different

TABLE II

VARIATIONS IN LIQUID AND LEAVENING MADE IN THE BASIC FORMULA

Group	Ingredients	Amount
I. Different liquids	Fresh whole milk	240 ml
	Evaporated milk plus water (distilled)	120 ml 120 ml
	Dry whole milk[1] plus water (distilled)	29 g 214 ml
	Water (distilled)	214 ml
II. Different types of baking powder	Sodium aluminum sulfate-phosphate baking powder (3 brands)	12.3 g[2]
	Phosphate baking powder	19.3 g[2]
	Tartrate baking powder	22.8 g[2]
III. Different amounts of baking powder	Sodium aluminum sulfate-phosphate baking powder	12.3 g[2]
	Sodium aluminum sulfate-phosphate baking powder	18.4 g
IV. Constant amount of lactic acid with varying amounts of baking soda[3]	Lactic acid Sodium bicarbonate Sodium aluminum sulfate-phosphate baking powder	2.05 g[4] 1.64 g[5] 10.28 g
	Lactic acid Sodium bicarbonate Sodium aluminum sulfate-phosphate baking powder	2.05 g 2.50 g 6.54 g
	Lactic acid Sodium bicarbonate Sodium aluminum sulfate-phosphate baking powder	2.05 g 3.00 g 4.52 g
	Lactic acid Sodium bicarbonate Sodium aluminum sulfate-phosphate baking powder	2.05 g 3.50 g 2.16 g
	Lactic acid Sodium bicarbonate Sodium aluminum sulfate-phosphate baking powder	2.05 g 4.50 g 0.00 g

[1] Whenever dry whole milk was used it was mixed with the dry ingredients.

[2] This amount is that recommended by the manufacturer for use with the amount of flour in the basic recipe.

[3] 29 g of dry whole milk plus 214 ml distilled water were used in these doughs. The amount of baking powder was varied to keep the total leavening as nearly constant as possible.

[4] This amount of lactic acid approximates that in 240 ml of full soured milk.

[5] This amount of soda is chemically equivalent to the lactic acid used.

positions were numbered according to the scheme in Figure 1. Four of the biscuits (Nos. 1, 5, 9, 10) were used for subjective judgment, four (Nos. 3, 4, 8, and 12) were used for objective measurements, and four (Nos. 2, 6, 7, 11) were used for thiamine, dry matter, and pH determinations.

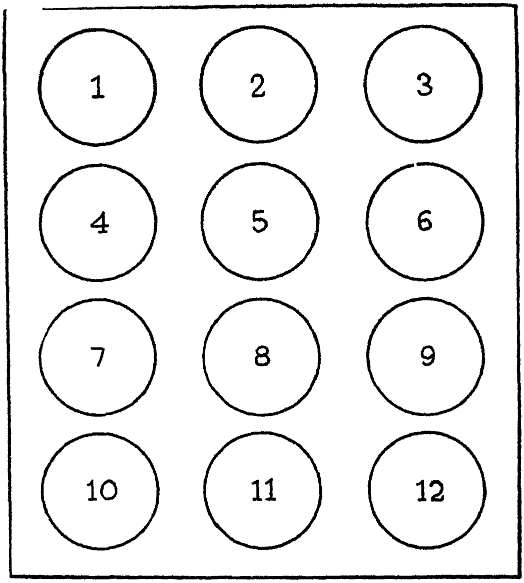


Fig 1. The position of the biscuits relative to each other.

Chemical Determinations

Sampling Methods. The biscuits were sampled 15 minutes after they were taken from the oven. Two opposite quarters of biscuit number 2 and two of biscuit number 6 were ground together in a mortar until fairly fine and crust and crumb were intimately mixed. One sample of this mixture was taken for each of the determinations of thiamine, dry matter, and pH. The procedure was repeated for biscuits 7 and 11.

Thiamine, dry matter, and pH of the dough were also determined. Dough samples were taken and weighed immediately after the biscuits had been cut. One sample for each determination was taken from around positions 2 and 6 and a second from around positions 7 and 11. The dough samples were taken from the positions adjacent to the biscuits used for similar determinations because preliminary experiments had shown this lessened the chance of large differences in thiamine and pH values between the dough used for the determinations and that in the biscuits.

Thiamine in the flour and milk was also determined, and these values were used in calculating the amount of thiamine in the doughs.

Analytical Methods. Thiamine was determined by a thiochrome method, essentially that of Conner and Straub (1941). All samples were blended with the acid immediately after being weighed. Dry matter was determined by the method of the American Association of Cereal Chemists (1941). A Beckman pH meter was used to measure pH. The determinations were made on the undiluted dough and on a slurry of one part of ground biscuit to two parts distilled water (McKim and Moss, 1943). Preliminary experiments had shown the error of measurement on the undiluted dough to be small. Determinations of pH could not be made on the undiluted biscuit.

Subjective judgments were made by three experienced judges on the hot biscuits 10 minutes after being taken from the oven. The biscuits were scored for external and internal color, tenderness of crust and crumb, flakiness, cell size, moisture, and flavor. The maximum score was 100.

Objective measurements were made after the biscuits had cooled to room temperature. The height of the four biscuits set one on top of the other was measured in centimeters.

Compressibility of the crumb was measured using the Baker compressimeter[1] described by Platt and Powers (1940). A one-centimeter slice was cut from the bottom of the biscuit. The effect of the side crust was eliminated by cutting out a two-inch disc from the center of this slice and using this for the measurement of compressibility.

The breaking strength of the bottom crust was measured using an instrument similar in principle to the Bailey shortometer (1934) in which weight is added gradually to a blunt knife-edge pressing down on the piece to be broken. The addition of weight ceased abruptly when the crust was broken, and this weight was recorded as "breaking strength."

Results and Discussion

The results of the complete study are shown in Table III. Each figure is the average of the values obtained on five batches. The coefficients of variability were 5% or below for thiamine retention, measurement of height, and judges' scores, and were from 10 to 20% for the hydrogen ion concentration, thiamine content, compressibility, and breaking strength.

The thiamine content of the flour and its water absorption capacity varied with the different bags in spite of the fact that all four bags

---

[1] The authors are indebted to the Borden Company, Syracuse, New York, for the loan of the compressimeter.

TABLE III

THIAMINE RETENTION, PH, AND QUALITY OF BISCUITS
(Averages for 5 batches)

Group	Variable	Thiamine[1]				pH		Objective measurements			Palatability score[5]
		Dough		Biscuit		Dough	Biscuit	Compressibility[4]	Breaking strength	Height of four biscuits	
		Calculated[2]	Found	Found	Retention[3]						
		µg/g	µg/g	µg/g	%			mm	g	cm	
I. Different liquids	Fresh milk	3.5	3.3	3.1	89	7.0	6.8	1.69	365	15.6	92
	Evaporated milk	3.4	3.2	2.8	84	7.1	6.6	1.43	309	13.2	80
	Dry milk plus water	3.5	3.2	2.9	83	7.2	6.7	1.47	346	13.2	81
	Water	3.6	3.2	2.9	81	7.5	6.9	1.47	378	13.2	81
II. Different types of baking powder	Tartrate	2.9	3.0	2.1	72	6.4	6.3	1.23	221	12.7	83
	Phosphate	2.9	2.8	2.2	75	6.7	7.0	1.11	244	13.5	86
	Sodium aluminum sulfate-phosphate Brand No. 1	2.9	3.0	2.5	84	7.0	6.8	1.25	280	14.7	89
	2	2.9	3.1	2.6	89	7.0	7.0	1.18	274	14.6	86
	3	2.9	2.9	2.4	83	7.1	6.8	0.99	284	14.4	89
III. Different amounts of baking powder	Sodium aluminum sulfate-phosphate Brand No. 3 12.3 g	3.0	2.9	2.6	86	7.0	6.9	1.03	263	13.7	84
	18.4 g	3.0	3.1	2.4	80	7.2	7.1	1.04	251	14.2	79
IV. Different proportions of bicarbonate of soda and baking powder, lactic acid being constant at 2.05 g	Soda 1.64 g Baking powder 10.28 g	3.3	3.3	2.8	84	7.0	7.0	1.03	324	13.2	86
	Soda 2.50 g Baking powder 6.54 g	3.3	3.2	2.3	70	7.2	7.5	1.03	326	12.5	80
	Soda 3.00 g Baking powder 4.52 g	3.4	3.2	1.6	48	7.2	8.0	0.99	359	11.9	76
	Soda 3.50 g Baking powder 2.16 g	3.4	3.2	1.0	30	7.3	8.1	1.04	399	12.1	70
	Soda 4.50 g Baking powder 0.00 g	3.4	3.0	0.5	15	7.4	8.6	1.13	392	11.2	63

[1] Expressed as micrograms of thiamine per gram of dry matter.
[2] Calculations are based on the amount of thiamine found in the flour and in the milk.
[3] Expressed as percent of the calculated amount of thiamine in the dough.
[4] The displacement of the plunger for a stress of 28 g.
[5] Maximum score 100.

were from the same mill lot and were stored together until used. These differences are reflected in all of the values for the fresh milk biscuits in groups 1, 2, and 3. The only difference in ingredients for these biscuits was in the flour.

Retention of Thiamine. The thiamine retained in the doughs was practically 100% of that calculated, even in biscuits with excess of soda. For the biscuits made with the different liquids, the difference between the extreme values for retention of thiamine is not significant.[2]

The thiamine retained in the biscuits made with the phosphate and the tartrate powders was significantly less than that in the ones made with any of the sodium aluminum sulfate-phosphate baking powders. These results are the reverse of those reported by Williams and Fieger (1944), due perhaps to a lower and unvarying proportion of baking powder to flour in the biscuits of Williams and Fieger. With a 50% increase in the amount of sodium aluminum sulfate-phosphate baking powder, there was a significant drop in the retention of thiamine.

The thiamine retained in the biscuits decreased significantly with each increase in the amount of soda when the leavening was derived from a constant amount of lactic acid with equivalent and excessive amounts of baking soda.

pH of Dough and Biscuit. The pH of the doughs varied little except when tartrate or phosphate baking powder was used. In these doughs the pH values were significantly lower than in the others. It should be remembered that the difference between pH 7.0 and 7.5 is much less in terms of hydrogen ion concentration than is the difference between pH 6.7 and 7.0.

The greatest change in pH with baking occurred in the biscuits made with the different proportion of lactic acid and soda. With increasing amounts of soda, the pH of the baked biscuit increased progressively. This increase was 1.2 pH units with the largest amounts of soda.

Barackman (1942) states that ". . . most baking powders result in biscuits considerably above 7.0 pH." The results in Table III do not bear out this statement. All biscuits leavened with baking powder at the level recommended by the manufacturer were found to have a pH of 7.0 or below. Even with a 50% increase in the amount of baking powder of the sodium aluminum sulfate-phosphate type, the pH of the baked product was only 7.1.

pH and Thiamine Retention. From the figures in Table III, it will be seen that (except for the tartrate and phosphate baking powders) when the pH of the biscuit was close to 7.0, 80% or more of the thiamine was retained in the baked product.

---

[2] Throughout this discussion, differences are reported as significant if, using Student's "t" test, the odds are 99:1 or greater.

Where the pH increased as the result of increase in the proportion of soda to acid ingredient or of increase in amount of baking powder, the retention of thiamine dropped. This agrees with results obtained on thiamine in pure solution (Beadle, Greenwood, and Kraybill, 1943), and with those of Barackman (1942). Farrer's (1945) experiments with thiamine in different salt solutions indicate that different buffer salts may influence the rate of thermal destruction of thiamine, and this may account for the lower retentions with the phosphate and tartrate baking powders. Melnick's results (1945) with thiamine in suspensions of baking powders are in agreement with this hypothesis.

Quality of Doughs. The different baking powders had a marked effect on the physical condition of the dough. With the tartrate powder, the dough was spongy and sticky; with the phosphate powder, the dough was somewhat spongy and sticky, but not as markedly so as with the tartrate powder. With the sodium aluminum sulfate-phosphate powders, the doughs were compact and were not sticky. The effect of these differences on external and internal appearance of the biscuits is shown in Figure 2. The doughs made with the different liquids exhibited only slight differences in physical condition and those made with the different amounts of soda and baking powder did not vary sufficiently to be detected during the manipulation.

Quality of Biscuits. The values for compressibility, breaking strength of the bottom crust, and height of four biscuits indicate that the flour from the different bags had more effect upon these factors than any other variable. It is pertinent, therefore, to discuss the differences in the values within groups rather than between groups. For the same reason, palatability scores will be considered only within groups.

The fresh milk biscuits in the first group were significantly more compressible than the others in the same group, which were similar to each other. There is no very significant difference between the breaking strength for the different types of biscuits. The fresh milk biscuits in this first group raised more than the others in the same group. The other biscuits were alike in their height.

The judges preferred the biscuits made with the whole milk. They found them more tender in crust and crumb and more pleasing in flavor than the other biscuits in this group. The total palatability score for the whole milk biscuits is significantly greater than that for any of the others in this group.

An increase of 50% in the amount of sodium aluminum sulfate-phosphate baking powder made no significant change in the compressibility, breaking strength, and height of the biscuit. The judges always scored the biscuits with the extra powder as significantly inferior to those made with the regular amount. These biscuits were poorer in external and internal appearance, in flavor, and were less flaky than those made with less baking powder.

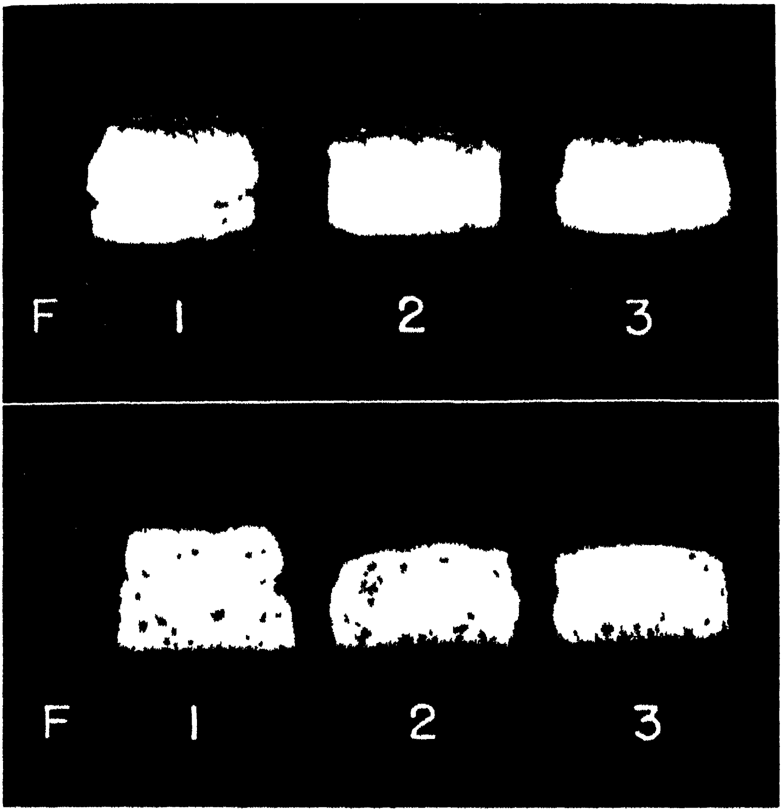


Fig. 2. External and internal appearance of biscuits made with different baking powders: 1, sodium aluminum sulfate-phosphate; 2, phosphate, 3 tartrate.

For the biscuits made with varying proportions of soda and baking powder, the compressibility did not vary with the changes in ingredients. The breaking strength, however, increased irregularly as the amount of soda was increased. The difference between the extreme values was significant, but was not significant between intermediate values. No explanation of this increase in toughness is offered. The height and the palatability of the biscuits decreased with increasing excess of soda to acid. The biscuits with soda at 3.00 g or more had a cream color, deepening with each increase in the amount of soda.

The judges were able to discern the flavor of soda at this and the higher levels. None of the biscuits were rated as unacceptable, however.

Summary

Biscuits were prepared using standardized formulae and preparation procedures. The effects of variation of ingredients on thiamine retention, pH, and quality of the biscuits were studied.

Biscuits made with fresh milk, evaporated milk, water, and dry whole milk plus water were alike in their thiamine content. The biscuits made with fresh whole milk retained slightly more of their thiamine than did the others. In eating quality, the biscuits made with fresh milk were judged superior; the others were equally acceptable to each other.

Biscuits made with sodium aluminum sulfate-phosphate baking powder (three brands) were alike in their thiamine content and retention. Increasing the amount of the baking powder by 50% (one brand) increased the loss of thiamine during baking. The biscuits made with a tartrate and with a phosphate baking powder lost more thiamine than those made with sodium aluminum sulfate-phosphate baking powders. All the biscuits were of similar eating quality.

Biscuits made with varying proportions of lactic acid, bicarbonate of soda, and baking powder retained less thiamine as the soda was increased. At the lowest level of soda 84%, and at the highest level of soda only 15%, of the thiamine was retained in the biscuits. The palatability of the biscuits decreased with each increment of soda, but none were inedible.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods. 4th ed. Lincoln, Neb.
Bailey, C. H.
1934 An automatic shortometer. Cereal Chem. 11: 160–163.
Barackman, R. A.
1942 Thiamin retention in self-rising flour biscuits. Cereal Chem. 19: 121–128.
Beadle, B. W., Greenwood, D. A., and Kraybill, H. R.
1943 Stability of thiamine to heat. I. Effect of pH and buffer salts in aqueous solutions. J. Biol. Chem. 149: 339–347.
Conner, R. T., and Straub, G. J.
1941 Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem. (Anal. Ed.) 13: 380–384.
Farrer, K. T. H.
1945 The thermal destruction of vitamin B_1. I. The influence of buffer salts on the rate of destruction of aneurin at 100°. Biochem. J. 39: 128–132.
McKim, E., and Moss, H. V.
1943 Observations on the pH of chemically leavened products. Cereal Chem. 20: 250–259.
Melnick, D., Oser, B. L., and Hochberg, M.
1945 Nutritional aspects of an improvement in baking powder formulation. J. Am. Dietet. Assoc. 21: 611–615.
Platt, W., and Powers, R.
1940 Compressibility of bread crumb. Cereal Chem. 17: 601–621.
Williams, V. R., and Fieger, E. A.
1944 Influence of ingredients on thiamine content of biscuits. Food Research 9: 328–337.

REPORT OF THE 1945-46 METHODS OF ANALYSIS SUBCOMMITTEE ON THIAMINE ASSAY

AARON ARNOLD

Sterling-Winthrop Research Institute,
Rensselaer, New York

(Presented at the Annual Meeting, May 1946, received for publication May 23, 1946)

The rapid methods for vitamin determination have come into favor among mill chemists who are faced with checking the enrichment level in flour during production. Towards evaluating and unifying current practice in the rapid methods for thiamine, a collaborative study was undertaken to compare the salient features of the published methods.

The rapid assay methods for thiamine in flour save time by eliminating the enzyme digestion and the base exchange steps of the A.A.C.C. thiamine method (*Cereal Laboratory Methods*, 1941; Andrews, 1944). This may be done in the analysis of flour since only a small fraction of the thiamine occurs bound as the pyrophosphate; on the other hand, the rapid methods for bread (Schiller, 1944) require the enzyme digestion step because of the appreciable content of bound vitamin.

Nicholls *et al.* (1942) and Glick (1944) successfully used dilute acid at room temperature to extract thiamine from flour. In contrast Andrews and Nordgren (1941), who first published a rapid thiamine method for flour, and Hoffer, Alcock, and Geddes (1943) observed acid potassium chloride solution to be an effective medium for extracting the vitamin. Although the latter two groups of investigators both used the same extracting solution, they differed as to the temperature at which the extraction was carried out; Andrews and Nordgren (1941) obtained satisfactory vitamin recovery at room temperature, while Hoffer and associates recommended extraction at elevated temperatures. Finally, Glick, and Andrews and Nordgren favored clarification of the extract by filtration, while Nicholls *et al.* and Hoffer and co-workers observed that extract clarification by centrifugation gave equally satisfactory thiamine extraction and was more rapid.

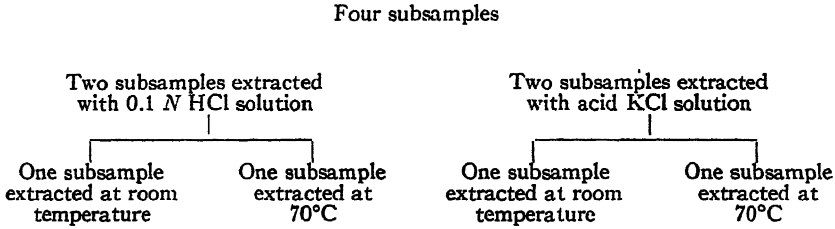
The points in the foregoing methods which appeared to require checking were (a) the type of extracting medium, (b) the temperature of extraction, and (c) the method of clarifying the extract. The procedure for the alkaline ferricyanide oxidation of thiamine to thiochrome is probably sufficiently well standardized so as not to require collaborative study.

With these points in mind, as well as the limitations of the amount of work which may be expected in collaborative study, the following paired procedure-steps were compared: 1. extraction with dilute acid vs. acid potassium chloride solution; 2. extraction at room temperature vs. elevated temperature; and 3. extract clarification by filtration vs. centrifugation.

While conclusions drawn from collaborative study are less subject to error when several samples are studied, only one sample was used in the present study in view of the several alternative points of procedure which were to be investigated.

Experimental

The collaborators were requested to analyze an A.A.C.C. National Check Sample of flour,[1] and also this sample to which was added 2 μg of thiamine per gram of sample[2] by the following variations in procedure:


Four subsamples
Two subsamples extracted with 0.1 N HCl solution
Two subsamples extracted with acid KCl solution
One subsample extracted at room temperature
One subsample extracted at 70°C
One subsample extracted at room temperature
One subsample extracted at 70°C

The directions supplied for conducting the analyses were essentially as follows:

Place the selected quantity of flour (2.0 g is suggested for the present study) in a 125 or 250 ml Erlenmeyer flask, and add 50 ml of 0.1 *N* hydrochloric acid or 50 ml of 25% potassium chloride solution acidified with 2% acetic acid or 0.1 *N* hydrochloric acid. Shake the uniformly suspended sample for 30 minutes in an automatic shaking device or intermittently by hand (incubate four of the portions at room temperature and the remaining four at 70°C). At the end of this time centrifuge the samples until the supernatant liquid contains only a negligible amount of suspended matter or filter them (pour the first portion of the filtrate back through the filter).

For the oxidation of thiamine to thiochrome, rapidly add 3 ml of 15% sodium hydroxide solution to a 5 ml aliquot of the clarified extract in a centrifuge, separatory funnel, or equivalent device, mix the contents, add 2 drops of 1% potassium ferricyanide solution, and again mix rapidly and uniformly. After 1 minute add 13 to 16 ml of isobutanol (the exact volume being determined by the routine practice in the collaborator's laboratory), shake the contents for 1 minute, centrifuge, and draw off the water layer. Clarify the alcohol layer by adding 1 ml of ethanol and estimate the fluorescence in the usual way. The attention of the collaborators was called to the fact that some time is saved by storing the isobutanol extracts in the cuvettes until the full series is at hand for reading (see Andrews, 1944).

---

[1] Samples prepared for mailing were supplied by W. R. Urban, Chairman of the A.A.C.C. Check Sample Committee. This was most useful, further, in that the collaborators were not required to test the sample by the A.A.C.C. procedure since the Check Sample value was already at hand.

[2] To reduce the possibility of differences developing owing to variations in the amount of thiamine added, ampules of thiamine hydrochloride solution at a concentration of 0.5 mg/ml were provided. It was directed that after appropriate dilution with either dilute acid for the one series or with acid potassium chloride solution for the second, a portion of the diluted thiamine hydrochloride solution be compared with the collaborator's own thiamine standard.

Express the results in µg of thiamine per gram of sample.

Results and Discussion

The results of the study, in which 16 laboratories participated, are summarized in Table I. At the outset it may be stated that the results of the study are in accord with the conclusions of Hoffer, Alcock, and

TABLE I

SUMMARY OF RESULTS OF THE THIAMINE CONTENT OF A COLLABORATIVE FLOUR SAMPLE AND OF THE SAMPLE WITH 2 µG OF ADDED THIAMINE BY SEVERAL MODIFICATIONS OF A RAPID METHOD

Extraction medium	Extraction Temp.	Method of extract clarification							
		Centrifugation (9 collaborators)				Filtration (8 collaborators)			
		No. of det'ns.	Thiamine content[1,2] µg/g			No. of det'ns.	Thiamine content[1,2] µg/g		
			Range	Mean[2] ± S.E.	Combined mean ± S.E.		Range	Mean[2] ± S.E.	Combined mean ± S.E.
COLLABORATIVE FLOUR SAMPLE									
Dilute acid	R.T.	19	3.37–4.31	3.71 ± .06	3.71 ± .04	20	3.07–4.01	3.59 ± .05	3.63 ± .01
	70°C	19	3.33–4.21	3.78 ± .06		19	3.07–4.19	3.67 ± .07	
Acid KCl	R.T.	19	3.63–4.41	3.92 ± .06	3.96 ± .01	22	3.52–4.38	3.81 ± .06	3.88 ± .01
	70°C	19	3.68–4.57	4.00 ± .07		19	3.61–4.59	3.96 ± .06	
COLLABORATIVE FLOUR SAMPLE WITH ADDED THIAMINE, 2 µG/G FLOUR									
Dilute acid	R.T.	19	5.22–6.14	5.52 ± .08	5.60 ± .05	20	5.28–6.35	5.56 ± .07	5.60 ± .05
	70°C	19	5.20–6.14	5.68 ± .07		19	5.14–6.35	5.64 ± .08	
Acid KCl	R.T.	19	5.00–6.97	5.99 ± .1	6.07 ± .07	19	5.43–6.58	6.00 ± .08	6.04 ± .06
	70°C	20	4.75–7.04	6.15 ± .09		19	5.54–6.85	6.07 ± .08	

[1] The thiamine content of the low-protein flour sample used in this study was approximately 4.12 µg/g, the mean reported by the Check Sample Service Committee.
[2] These thiamine values were calculated in terms of an external thiamine standard.

standards.

Geddes (1943). As reviewed above, these investigators recommended sample extraction with acid potassium chloride solution at elevated temperature followed by centrifugation of the extract. The discussion in the following paragraphs gives the reasons for these conclusions.

Extraction Medium. There are data on eight pairs of samples in the table, two at room temperature and two at 70°C for the extracts clarified by centrifugation, and a like number for the extracts clarified by filtration. These data may be used to compare the completeness of extraction by dilute acid with that resulting from the use of acid potassium chloride solution. The combined means clearly show more thiamine was extracted by acid potassium chloride solution than by dilute acid in every instance. Comparable pairs differ only 6 to 8% but the results are consistent enough that statistical analysis shows that a difference of this magnitude would not occur by chance as often as once in a thousand trials. The data are thus interpreted as indicating that acid salt solution is the extraction medium of choice.

One of the collaborators reported that "the standard made up in acid potassium chloride solution apparently deteriorated so rapidly that no reading was obtained. The standard made up with 0.1 *N* hydrochloric acid, however, gave the expected results." The uncommon and unexplained difficulty encountered by this collaborator has not been reported by others.

Temperature of Extraction. The data in Table I yield eight pairs of analysis bearing on the comparison of the two temperature conditions for extraction. As in the comparison of the two extraction media, in every instance slightly more thiamine was extracted at elevated temperature than at room temperature.

The individual pairs do not differ significantly, however, nor is a statistically significant difference obtained when all the results bearing on this factor are combined into a single difference. When this is done, only 3% more thiamine appears to have been extracted at 70°C than at room temperature. Since chance alone was not likely to cause slightly better thiamine extraction at the higher temperature in a comparison of eight pairs, the results are interpreted to indicate that extraction at 70° C is to be preferred to extraction at room temperature.

One of the collaborators (J. H. Lanning) reported that extraction at room temperature is favored in his laboratory. This is done to avoid the possible slight errors which may result when aliquots of solutions at a temperature appreciably different from 20°C are measured out. Changes in volumes due to evaporation are also avoided. In view of the slightly better vitamin extraction at the higher temperature it is possible that errors are more than compensated for when due to the foregoing reasons.

Method of Extract Clarification. The data given in the table likewise bear on the problem of the method of clarification of the extracts. Fortunately, there was an even division among the collaborators, eight utilizing filtration to clarify the extracts and nine utilizing centrifugation. The results of six of the eight pairs show slightly better thiamine extraction when the extracts were clarified by centrifuging. Only two of the results show better thiamine extraction when the extracts were clarified by filtering them. Considering that the object of the rapid method is to save time, these data strongly support clarification of the extracts by centrifugation. Using the centrifuge not only saves considerable time but also gives, on the average, a result slightly nearer that obtained by the A.A.C.C. method.

Several collaborators mentioned that extraction with acid potassium chloride solution yielded clearer extracts by centrifugation than did dilute acid extracts. The foregoing preferred procedures thus complement each other.

Thiamine Recoveries from the Samples with Added Thiamine. To determine the effect of the procedures on added vitamin, 2 μg of thiamine were added to portions of the sample by the collaborators. When acid potassium chloride solution was the extracting medium, slightly more thiamine than this appears to have been recovered. As judged by the combined means, the recovery was 2.13 to 2.16 μg of thiamine. This is simply taken to mean that slightly more of the thiamine in the sample itself was extracted from these portions since it was theoretically possible to obtain as much as 6.12 μg of thiamine from these samples (2 μg of thiamine added to a sample which was reported to contain 4.12 μg of thiamine per gram). The use of dilute acid as the extracting medium served to extract most of the added thiamine though not all; 1.86 to 1.97 μg of added thiamine recovered as judged by the combined means.

The over-all results do not favor the use of an internal thiamine standard, i.e., calculation of the thiamine content of the sample based on the fluorescence of the 2 μg thiamine increment added to a second portion of the sample, as a means of evaluating the thiamine content of the sample. Under conditions of good extraction, calculations of the thiamine content of the sample based on an external standard appear to yield reliable values. The slight upward correction factor recommended by Hoffer *et al.* (1943) corrects for the bound thiamine in the sample rather than for incomplete extraction of the free thiamine. This correction factor is somewhat dependent on the type of flour studied.

Oxidation Procedure. For the study, alcohol clarification of the isobutanol extract was suggested rather than the more usual clarification with anhydrous sodium sulfate. Recently Watson (1946) has also recommended alcohol clarification to avoid the possibility of fluorescence inaccuracies attributable to the anhydrous sodium sulfate. Several collaborators observed alcohol clarification to be unsatisfactory so that it did not meet with universal approval. With care the two procedures may probably be used interchangeably.

Similarly, the procedure as given followed Andrews' (1944) recommendations, recently confirmed by Watson (1946), regarding the successive addition of alkali and ferricyanide for the oxidation of thiamine to thiochrome. Collaborator objection was voiced regarding the use of these reagents separately rather than together. The use of these reagents separately does not prove burdensome once the procedure is established and is to be recommended on the basis that it yields results which appear to agree better with expected values.

One collaborator (D. Melnick) has mentioned that the use of an automatic pipette shaker, of a type on the market, to rock the separatory centrifuge funnels during the oxidation step eliminated the troublesome factor of lack of uniformity because of shaking Two funnels are readily shaken at a time by constructing a simple adapter for the shaker.

Summary

On the basis of the means of the results of the 1945–46 collaborative study, in which 16 laboratories participated, the following conclusions are drawn:

1. Extraction with acid potassium chloride solution appears to extract significantly more thiamine than does extraction with dilute acid. 2. Extraction at 70°C appears to be slightly more effective than room temperature for extracting thiamine from flour samples. 3. Extract clarification is accomplished more rapidly and with at least as good thiamine recovery by centrifugation as by filtration. 4. Extraction of added thiamine was complete by the preferred procedures so that confirmation of their effectiveness, as judged by study with the particular sample submitted to the collaborators, was obtained.

Acknowledgments

The writer wishes to express his appreciation to Mrs. Mary Lenz for the statistical treatment of the data and to the following who participated in the collaborative study: A. W. Alcock, Purity Flour Mills, Winnipeg, Manitoba; H. J. Alleman, The Kroger Food Foundation, Cincinnati, Ohio; H. M. Boyd, General Mills, Minneapolis, Minnesota; L. J. Daniel, Cornell University, Ithaca, New York; C. F. Davis, Schwarz Laboratories, New York, N. Y.; W. F. Geddes, University of Minnesota, St. Paul, Minnesota; R. L. Gray, Novadel-Agene Corporation, Buffalo, New York; L. W. Haas, The W. E. Long Company, Chicago, Illinois; C. Hoffman, Ward Baking Company, New York, N. Y.; A. J. King, Fisher Flouring Mills Company, Seattle, Washington; J. H. Lanning, Continental Baking Co., Jamaica, New York; E. Lerner, Lab-

oratory of Vitamin Technology, Chicago, Illinois; D. Melnick, Food Research Laboratories, Long Island City, New York; M. D. Mize, Wallace and Tiernan Company, Chicago, Illinois; J. H. Panton, Winthrop Chemical Company, Rensselaer, New York; W. R. Urban, Omaha Grain Exchange Laboratories, Omaha, Nebraska; W. H. Ziemke, Quality Bakers of America, New York, N. Y.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods. 4th ed.
Andrews, J. S.
1944 Report of the 1943-44 methods of analysis subcommittee on thiamine assay. Cereal Chem. **21**: 388-397.
——, and Nordgren, R.
1941 The application of the thiochrome method to the thiamin analysis of cereals and cereal products. Cereal Chem. **18**: 686–695.
Glick, D.
1944 Simplified procedures for the determination of thiamine in wheat flours and bread by the thiochrome method. Cereal Chem. **21**: 119–126.
Hoffer, A., Alcock, A. W., and Geddes, W. F.
1943 A rapid method for the determination of thiamine in wheat and flour. Cereal Chem. **20**: 717-729.
Nicholls, J. R., Booth, R. G., Kent-Jones, D. W., Amos, A. J., and Ward, H. H.
1942 Vitamin B_1 (aneurine) assay in white flour. Analyst **67**: 15-17.
Schiller, G. W.
1944 Simplification of the thiochrome method for thiamine determination. Cereal Chem. **21**: 514-518.
Watson, H. A.
1946 Study of some factors influencing the oxidation of thiamine to thiochrome. Cereal Chem. **23**: 166-177.

BOOK REVIEWS

Hackh's Chemical Dictionary. By Julius Grant. 925 pp. The Blakiston Company, Philadelphia, Pa. 1946. Price $8.50.

The third edition of this well-known and authoritative chemical dictionary was published in 1944 and reprinted in 1946 with some changes and additions. It contains some 57,000 entries and not only covers the terms used in chemistry and a brief statement of chemical theories and laws but also includes the vocabularies of physics, geology, mineralogy, botany, zoology, medicine, and pharmacy. Many of the terms used in industry, mining, and commerce are included.

The properties of the elements and a large number of compounds are described. Compounds are listed and described in systematic order, including the name, formula, molecular weight, synonyms, occurrence, preparation or type of substance, appearance, physical properties, as well as chemical, industrial, and medical uses. Various types of chemical apparatus are listed and brief biographies with portraits are given of scientists who have made important contributions to the progress of chemistry.

This book contains such a large body of classified information in the field of chemistry and related sciences that it will prove to be a valuable addition to any chemist's library.

W. F. Geddes
Division of Agricultural Biochemistry
University of Minnesota
St. Paul 1, Minnesota

Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Sixth Edition. 932 + xii pages, 76 illustrations, and 31 reference tables. Published by the A.O.A.C., Box 540, Benjamin Franklin Station, Washington 4, D. C. 1945. Price per copy domestic $6.25, foreign $6.75. ($5.00 to members when ordered through the office of the Association.) If five or more copies are ordered at one time for delivery to a single address, the price per copy is $5.00 domestic and $5.40 foreign.

The most recent revision of this compilation of methods again offers to analysts a wealth of information on procedures for use with agricultural, food, and drug products. Most of the material of the fifth edition has been retained, and to it substantial additions have been made—as witness the increase of nearly 200 pages over the previous edition. Five new chapters have been added—on cosmetics, enzymes, gelatin products, vitamins, and extraneous materials in foods and drugs.

Of the new chapters, those on vitamins and on extraneous materials represent the most significant additions and will be of chief interest to the cereal chemist. Included in the former chapter are familiar techniques for thiamine and riboflavin, as well as procedures for the estimation of vitamin A and carotene, for niacin (by microbiological means), and for vitamins D and K. The chapter on extraneous materials will be welcomed by all food and drug processors, embodying as it does a number of tested procedures useful in maintaining a high standard of plant sanitation.

Cross references again abound—a style which is doubtless economical of space, but which certainly does not facilitate ready use. It is still usually necessary in looking up an unfamiliar method to assemble the complete information from at least two, and often more, isolated paragraphs, and to add whatever operating notes may be necessary for best results. Brevity of presentation is often commendable, but on other occasions may lead to some difficulty. Thus in the determination of DDT by the labile chloride method, no mention is made of the fact that presence of chlorides from any other source will invalidate results. This fact will be apparent to the thoughtful analyst who considers the chemical basis of the procedure, but is not readily evident from the text. Other similar examples are numerous.

Printing and binding are well done, and the book appears reasonably free from typographical errors. Again this treatise will find a welcome place on the reference shelves of most cereal laboratories.

F. C. Hildebrand
General Mills, Inc.
Minneapolis, Minnesota

The Baking Industry. By Mary Kinnavey Moore. 32 pp. Billman Publishing Co., Inc., Boston, Mass. 1946. Price $1.00.

The baking industry, the country's largest food industry in number of employees and payroll size and the world's oldest manufacturing occupation, has followed closely the advancement of agriculture. Starting with home baking and following the trend established by industrial revolution, baking expanded into neighborhood shops and then into large wholesalers. Statistics are quoted as to the dollar value of the industry and as to the consumption of flour per capita.

The classifications of the baking industry are given in detail—wholesale, retail, and house-to-house operations.

The subdivisions of baking are thoroughly explained, from bread production through sales.

Anyone in the baking industry will be interested in Part IV on personnel. This is a very good outline of the duties of each person employed in the baking business.

Statistics are given as to wages earned by those employed in this industry; 201,533 employees earned $262,001,562 per year. Workers can choose between the handcraft production in small shops or machine production of large plants, and occupational hazards are few.

This is a good review of the baking industry.

W. A. Richards
Russell-Miller Milling Company
Minneapolis, Minnesota

CEREAL CHEMISTRY

VOL. XXIII NOVEMBER, 1946 No. 6

EFFECT OF PARBOILING ON THIAMINE IN RICE[1]

M. C. KIK

Agricultural Chemistry Department, University of Arkansas,
Fayetteville, Arkansas

(Received for publication June 25, 1946)

The process of parboiling consists essentially of soaking rough rice in cold, warm, or hot water followed by draining the water, steaming the rice, and drying. The hypothesis is that during such treatment the water-soluble vitamins diffuse from the outer layers of the kernel to the inner layers and more of these water-soluble nutrients are retained in the kernel.

The parboiling, as practiced in the Orient, is by no means standardized. In Malaya (Jack, 1923), the paddy is merely heated by steam under pressure, quickly dried, and milled. In 1935 Jones and Taylor concluded from their experimental studies that only 12 hours of soaking at 60°–70°C, followed by steaming, are required to give a good product. A review of these methods is given by Kik (1945).

Modern commercial practices of parboiling find application in the process of rice conversion, in the Malek parboiling process (Kik and Williams, 1945), and in the Sacramento Mill in California where rough rice is soaked and exposed to steam under pressure.

Data have been published on the effect of parboiling and milling on the thiamine content of rice (Aykroyd, 1932) and on milling quality (Jones and Taylor, 1935; Subrahmanyan, Sreenivasan, and Das Gupta, 1938). No extensive study has been made of the thiamine content of rice which has been parboiled according to simple controlled methods in which modern analytical procedures for the determination of the thiamine content have been employed.

This investigation deals with the effect of simple controlled methods of parboiling of rough rice on the thiamine content of the milled form after approximately the same percent of the bran (10%) has been removed. Shelling tests were made on all these samples, which showed the breakage during shelling. Data are also presented on the effect of soaking for different lengths of time on the thiamine content of milled rough and brown rice and of losses of this vitamin in the soaking water.

---

[1] Research paper No. 813, Journal Series, University of Arkansas. Published with the permission of the Director of the Arkansas Agricultural Experiment Station. Aided by grants from the Williams-Waterman Fund of the Research Corporation and of Converted Rice. A preliminary report has been presented at the meeting of the American Institute of Nutrition, which was held in Atlantic City, New Jersey, on March 11–15, 1946.

Materials and Methods

A total of 88 samples (weight 60 g) of the same lot of previously cleaned rough rice, medium grain (Zenith variety), were soaked in 150 ml of distilled water under varying conditions of duration and temperature and with variations in amounts of steam pressure. These samples of rough rice were parboiled by boiling; boiling and steaming; steaming; soaking and steaming; soaking, boiling, and steaming; according to rice conversion with vacuum; and according to rice conversion without vacuum. The details of these treatments are given in Tables I and II. After the treatment, the water was drained off,

TABLE I

EFFECT OF DIFFERENT METHODS OF PARBOILING ROUGH RICE (ZENITH VARIETY) ON THIAMINE[1] IN MILLED RICE AND ON BREAKAGE[2] DURING SHELLING
(Milled to approximately 10% bran removal)

Sample No	Treatment	Thiamine	Appearance of the milled product	Breakage
		μg/g		%
1	No treatment	0.66	Opaque	3.6
2	No treatment	0.40	Opaque	4.1
3	No treatment	0.79	Opaque	3.6
4	No treatment	0.56	Opaque	3.9
	Average	0.60		3.8
	a. *Boiling:*			
5	For 5 minutes	2.39	Opaque	45.2
6	For 10 minutes	2.31	Opaque	40.6
7	For 15 minutes	2.24	Opaque	27.6
	Average	2.31		37.8
	b. *Boiling and Steaming:*			
8	Boiled 5 min., steamed 10 min. at 5 lbs. pres.	2.40	Opaque	6.0
9	Boiled 5 min., steamed 10 min. at 10 lbs. pres.	2.14	Opaque	6.0
10	Boiled 5 min., steamed 10 min. at 15 lbs. pres.	2.04	Opaque	10.2
11	Boiled 10 min., steamed 10 min. at 5 lbs. pres.	2.36	Opaque	12.0
12	Boiled 10 min., steamed 10 min. at 10 lbs. pres.	2.25	Opaque	13.8
13	Boiled 10 min., steamed 10 min. at 15 lbs. pres.	2.00	Partly translucent	2.4
14	Boiled 10 min., steamed 15 min. at 10 lbs. pres.	2.34	Opaque	2.6
15	Boiled 15 min., steamed 15 min. at 10 lbs. pres.	2.61	Opaque	4.4
16	Boiled 20 min., steamed 15 min. at 10 lbs. pres.	2.13	Translucent	4.0
	Average	2.25		6.8

TABLE I—(*Continued*)

Sample No.	Treatment	Thiamine	Appearance of the milled product	Breakage
		μg/g		%
	c. *Steaming:*			
17	10 minutes at 5 lbs. pres.	2.36	Opaque	27.1
18	20 minutes at 5 lbs. pres.	2.43	Opaque	13.2
19	30 minutes at 5 lbs. pres.	2.44	Opaque	9.7
20	5 minutes at 10 lbs. pres.	1.96	Opaque	11.3
21	10 minutes at 10 lbs. pres.	2.05	Opaque	10.1
22	15 minutes at 10 lbs. pres.	2.27	Opaque	3.5
23	20 minutes at 10 lbs. pres.	2.40	Opaque	7.6
24	5 minutes at 15 lbs. pres.	2.40	Opaque	52.7
25	10 minutes at 15 lbs. pres.	2.33	Opaque	29.2
26	20 minutes at 15 lbs. pres.	2.54	Opaque	8.4
27	5 minutes at 20 lbs. pres.	2.46	Opaque	52.6
28	10 minutes at 20 lbs. pres.	2.40	Opaque	8.5
29	15 minutes at 20 lbs. pres.	2.19	Opaque	4.8
	Average	2.51		
	d. *Soaking, Boiling, and Steaming:*			
83	2 hours soaking at 27°C, boiled 5 min., steamed 10 min. at 15 lbs. pres.	2.34	Opaque	15.1
84	2 hours soaking at 27°C, boiled 10 min., steamed 10 min. at 10 lbs. pres.	2.32	Opaque	46.7
85	4 hours soaking at 27°C, boiled 5 min., steamed 10 min. at 15 lbs. pres.	2.01	Translucent	2.0
86	4 hours soaking at 27°C, boiled 10 min., steamed 10 min. at 15 lbs. pres.	2.30	Translucent	2.5
	e. According to the principle of rice conversion:			
87	30 min. vacuum, pressure 60 lbs./inch at 70°C for 2½ hours.	2.48	Translucent	3.5
88	No vacuum, pressure 60 lbs. at 70°C for 2½ hours.	2.35	Translucent	3.5

[1] Expressed on a basis of 9% moisture. [2] Determined with Smith's Shelling Device.

the rough rice was dried overnight at room temperature with the aid of a fan, shelled, milled to approximately 10% bran removal, and assayed for thiamine. Four nontreated samples were used as controls.

In the final evaluation of the efficiency of the parboiling process, the appearance of the rice kernel after milling and the percentage of breakage during shelling, as well as the retention of thiamine in the milled product, have to be taken into consideration, since these properties are of paramount economic importance in the industrial application of these results.

The shelling and milling were done with laboratory-scale equipment. The seed huller[2] was used for the removal of hulls from the rough rice, and the small hand barley pearler [2] was employed for the milling of brown rice to 10% bran removal.

---

[2] Purchased from the Seedburo Equipment Company, 223 West Jackson Boulevard, Chicago 6, Illinois.

The rice was steamed under pressure in a small pressure cooker. The pressure ranged from 5 to 20 pounds and the temperature from 104°C to 127°C. The laboratory processing of rice according to the

TABLE II

EFFECT OF SOAKING AND STEAMING OF ROUGH RICE (ZENITH VARIETY) ON THIAMINE IN MILLED RICE AND ON BREAKAGE DURING SHELLING

(Milled to approximately 10% bran removal)

Sample No.	Treatment	Thiamine	Appearance of the milled product	Breakage
		µg/g		%
	a. Soaking for 15 hours at room temperature (27°C) followed by:			
30	5 min. steaming at 15 lbs. pres.	2.48	Partly translucent	3.5
31	10 min. steaming at 15 lbs. pres.	2.14	Translucent	6.1
32	15 min. steaming at 15 lbs. pres.	2.29	Translucent	5.2
33	20 min. steaming at 15 lbs. pres.	2.13	Translucent	9.1
34	25 min. steaming at 15 lbs. pres.	2.71	Translucent	6.1
35	30 min. steaming at 15 lbs. pres.	2.22	Translucent	8.8
36	35 min. steaming at 15 lbs. pres.	2.32	Translucent	6.7
37	40 min. steaming at 15 lbs. pres.	2.00	Translucent	3.2
	Average	2.28		6.1
	b. Soaking for 45 hours at room temperature (27°C) followed by:			
38	10 min. steaming at 15 lbs. pres.	2.25	Translucent	0.9
39	15 min. steaming at 15 lbs. pres.	2.26	Translucent	1.7
40	20 min. steaming at 15 lbs. pres.	2.25	Translucent	2.8
41	25 min. steaming at 15 lbs. pres.	1.97	Translucent	4.7
42	30 min. steaming at 15 lbs. pres.	1.73	Translucent	5.7
43	35 min. steaming at 15 lbs. pres.	1.99	Translucent	3.8
	Average	2.07		3.2
	c. Soaking for indicated length of time at 35°C, followed by 10 min. steaming at 15 lbs. pres.			
44	2 hours	1.93	Opaque	3.1
45	4 hours	2.21	Slightly opaque	3.0
46	6 hours	2.09	Translucent	3.3
47	8 hours	2.24	Translucent	6.5
48	10 hours	2.80	Translucent	2.9
49	12 hours	2.40	Translucent	3.8
50	16 hours	2.30	Translucent	2.1
51	20 hours	2.08	Translucent	2.3
52	24 hours	2.57	Translucent	1.0
53	36 hours	2.57	Translucent	2.7
	Average	2.29		3.1
	d. Soaking for 6 hours at 40°C followed by:			
54	15 min. steaming at 15 lbs. pressure (Malekized process)	2.25	Translucent	0.9

TABLE II—(*Continued*)

Sample No.	Treatment	Thiamine	Appearance of the milled product	Breakage
		μg/g		%
	e. Soaking for indicated length of time at 60°C, followed by 10 min. steaming at 15 lbs. pres.			
55	2 hours	1.91	Translucent	2.9
56	4 hours	2.27	Fine translucent	1.9
57	6 hours	2.16	Fine translucent	2.3
58	8 hours	2.16	Fine translucent	3.1
59	10 hours	2.07	Fine translucent	5.9
60	12 hours	2.28	Fine translucent	2.6
61	16 hours	2.31	Translucent	2.2
62	20 hours	2.26	Translucent	3.8
63	24 hours	2.27	Fine translucent	4.3
64	36 hours	1.93	Fine translucent	3.8
	Average	2.16		3.3
	f. Soaking for 2 hours at 70°C, followed by:			
65	5 min. steaming at 15 lbs. pres.	2.91	Opaque	6.1
66	10 min. steaming at 15 lbs. pres.	2.66	Slightly translucent	6.3
67	15 min. steaming at 15 lbs. pres.	2.72	Fair translucent	6.6
68	20 min. steaming at 15 lbs. pres.	2.64	Opaque	5.7
	Average	2.73		6.2
	g. Soaking for indicated time at 70°C, followed by: 10 min. steaming at 15 lbs. pres.			
69	1 hour	2.38	Opaque	2.3
70	2 hours	2.57	Slightly translucent	2.0
71	3 hours	2.46	Translucent	6.7
72	4 hours	2.88	Translucent	3.9
73	5 hours	2.48	Fine translucent	3.3
74	6 hours	2.34	Translucent	3.0
	Average	2.52		3.5
	h. Soaking for indicated time at 80°C, followed by: 10 min. steaming at 15 lbs. pres.			
75	½ hour	2.44	Slightly opaque	3.5
76	1 hour	2.48	Opaque	5.4
77	2 hours	2.58	Translucent	13.0
78	3 hours	2.29	Translucent	14.6
	Average	2.45		9.1
	i. Soaking for indicated time at 90°C, followed by: 10 min. steaming at 15 lbs. pres.			
79	10 minutes	2.52	Slightly translucent	3.6
80	20 minutes	2.51	Slightly translucent	10.3
81	30 minutes	2.10	Translucent	6.8
82	40 minutes	2.35	Translucent	4.9
	Average	2.37		6.4

principles of rice conversion in which 60 pounds pressure was applied has been described in a previous publication (Kik, 1945).

In soaking experiments, eleven 100-g samples of previously cleaned rough and brown rice (Zenith, medium grain variety) were soaked from 0 to 36 hours in 150 ml of distilled water. The rice was dried, shelled, milled to approximately 10% bran removal, and tested for thiamine by the thiochrome method, with the adaptation of the Hennessy and Cerecedo procedure (1939). The thiamine results are expressed on a basis of 9% moisture. The results of these tests are given in Table III.

TABLE III

EFFECT OF TIME OF SOAKING OF BROWN AND ROUGH RICE ON THE THIAMINE CONTENT OF MILLED RICE

Sample No.	Time of soaking	Average thiamine content of milled rice	Loss of thiamine in steep water
	hours	*µg/g*	*%*
SOAKING OF BROWN RICE			
1	0	0.44	0.00
2	8	1.03	10.90
3	12	1.08	14.24
4	24	0.91	15.80
5	36	0.79	16.00
SOAKING OF ROUGH RICE			
6	4	1.35	1.71
7	8	1.03	1.30
8	12	1.12	4.04
9	16	1.50	2.01
10	24	1.37	2.63
11	36	1.58	1.86

Results

Parboiling Tests. The data relating to parboiling tests are presented in Tables I and II. The nontreated controls, numbers 1, 2, 3, and 4, had an average content of thiamine of 0.60 µg/g and the average breakage on shelling was 3.8%.

BOILING: Three samples of rough rice (numbers 5, 6, 7) were exposed to boiling water for 5, 10, and 15 minutes respectively and showed a considerable retention of thiamine (average content 2.31 µg/g) which was not affected by the time of exposure to boiling water. However, excessive breakage (average 37.8%) occurred during shelling, which makes the treatment of boiling alone rather unsatisfactory. Exposure to boiling water for 15 minutes (sample 7) retarded the breakage to 27.6%. This showed that prolonged boiling helped reduce breakage.

BOILING AND STEAMING: A total of nine samples, numbers 8 to 16 inclusive, was used (see *b* Table I). Samples 8, 9, and 10 were boiled for 5 minutes followed by steaming for 10 minutes at pressures ranging from 5 to 15 pounds (109°C–121°C). The milled samples were opaque in appearance like ordinary milled rice, whereas parboiled rice is translucent to amber, and generally of a straw-yellow color. The translucence is due to the complete gelatinization of the starch which takes place at temperatures varying from 60° to 75°C (Jones and Taylor, 1935). Some of the kernels in these samples showed a white spot in the center of the grain due to partial gelatinization of the starch.

The additional steaming reduced the breakage from an average of 37.8% in boiling tests to 6.8% in boiling and steaming tests. This indicates that steaming decreases the breakage. The lowest breakage (2.4%) was obtained after the steam pressure was increased from 5 and 10 pounds (samples 11 and 12) to 15 pounds (sample 13). Fairly well parboiled rice was obtained in sample 16 which was boiled for 20 minutes and steamed afterwards for 15 minutes at 5 pounds pressure. The average thiamine content in these samples was 2.25 μg/g, which showed that additional steaming did not have any beneficial effect. Prolonged boiling did not have any effect on the thiamine content of the milled product.

STEAMING: The rough rice was previously soaked for 10 minutes in water of room temperature, as dry starch cannot be gelatinized and the kernels are brittle after steaming. A total of 13 samples was steamed at pressures ranging from 5 to 20 pounds for different lengths of time. The average thiamine content of the milled samples was 2.51 μg/g (see Table I). The breakage was appreciably reduced by proper steaming. A breakage of 27.1% was reduced to 9.7% when 10 minutes' steaming at 5 pounds pressure (sample 17) was increased to 30 minutes (sample 19). Similar comparisons can be made with samples 24 and 26, samples 27 and 29. All kernels were still opaque after the treatment, which indicates that steaming after only a brief soaking in water of room temperature does not give satisfactory results.

SOAKING AND STEAMING: *a.* Eight samples, numbers 30 to 37, were *soaked for 15 hours at room temperature (27°C).* The samples were steamed afterwards at 15 pounds pressure for a duration ranging from 5 minutes for sample 30 to 40 minutes for sample 37. The results are presented in Table II. The average thiamine content was 2.28 μg/g; the average breakage was 6.1%. All kernels were translucent except those of number 30. The best results were obtained in number 34 which was steamed 25 minutes at 15 pounds pressure.

b. Another six samples, numbers 38 to 43, were *soaked at room temperature* (27°C) for 45 hours (See *b* Table II). The samples were steamed at 15 pounds pressure for from 10 minutes for sample 38 to 35 minutes for sample 43. Nice translucent kernels were obtained which showed an average breakage of 3.2%. Number 38, which showed the best results, had a thiamine content of 2.25 µg/g and a breakage of 0.9%. This sample was steamed for 10 minutes at 15 pounds pressure and the kernels were fully translucent in the milled state. A small decrease in thiamine content and a small increase in breakage were found in those samples (41, 42, and 43) which were steamed more than 20 minutes. In separate tests it was found that thiamine was partly destroyed when exposed for over 20 minutes at 15 pounds pressure.

c. The next series of samples was *soaked 2, 4, 6, 8, 10, 12, 16, 20, 24, and 36 hours in water of 35°C* followed by steaming for 10 minutes at 15 pounds pressure. In all 10 cases (numbers 44 to 53) except number 44, translucent kernels were obtained which showed an average breakage on shelling of 3.1% and an average thiamine content of 2.28 µg/g. Good results were found in all samples after a minimum of 6 hours of soaking. Number 52 showed a thiamine content of 2.57 µg/g, a breakage of 1%, and translucent kernels after milling. This sample was soaked for 24 hours before the steam treatment was applied.

d. One sample, number 54, was *soaked for 6 hours at 40°C* followed by 15 minutes steaming at 15 pounds pressure. This method is applied in the Malekized process which is used on a commercial scale. The sample was milled to the same degree of bran removal as all other samples and showed a thiamine value of 2.25 µg/g. The kernels were translucent and an exceedingly low breakage of 0.9% was obtained during shelling.

e. The next series consisted of *10 samples which were soaked at 60°C for 2, 4, 6, 8, 10, 12, 16, 20, 24, and 36 hours respectively*. In all cases translucent kernels were found in the milled product. The average thiamine content in this series was 2.16 µg/g and the average breakage was 3.3%. However, best results were obtained from samples soaked a minimum of 4 hours.

f. Soaking for 2 hours at 70°C followed by steaming at 15 pounds pressure took place in four samples, numbers 65 to 68, which were steamed for 5, 10, 15, and 20 minutes. These kernels were not quite translucent after milling and the average breakage was 6.2%, which was higher than the 3.8% found for the nontreated controls. However, the average thiamine content was 2.73 µg/g, which is higher than has been found in milled samples exposed to other treatments.

g. Six samples were *soaked at 70°C followed by 10 minutes at 15 pounds pressure* for 1, 2, 3, and 6 hours respectively. The results were good after the rice had been soaked for more than 2 hours. This can be seen from Table II (samples 69, 70, 71, 72, 73, and 74). The first two samples showed kernels that were not entirely translucent. The remaining four samples were well parboiled. The average thiamine content of this group was 2.52 μg/g, with an average breakage of 3.5%. It would appear that soaking for 4 hours at 70°C, followed by 10 minutes steaming at 15 pounds pressure, gives the optimum results.

h. Four samples, 75, 76, 77, and 78, were *soaked for ½ hour, 1, 2, and 3 hours respectively at 80°C followed by 10 minutes steaming at 15 pounds pressure.* Translucent, well-parboiled kernels were obtained after 2 and 3 hours soaking at this high temperature. However, the breakage on shelling was quite high (13 and 14.6%) which might be reduced by a longer period of steaming, a higher pressure, or a more effective method of drying. The average thiamine content amounted to 2.45 μg/g.

i. Soaking at 90°C for 10, 20, 30, and 40 minutes, followed by 10 minutes steaming at 15 pounds pressure, was performed with samples numbered 79, 80, 81, and 82. The rice of the first two samples showed slightly translucent kernels. The kernels of the last two samples (81 and 82) were well parboiled, had an average of 2.22 μg/g of thiamine, and an average breakage of 5.9%.

SOAKING, BOILING, AND STEAMING: The combination of 2 hours soaking, boiling for 5 minutes, followed by steaming for 10 minutes at 15 pounds pressure, was used in sample 83 (see *d* Table I). A similar combination, using 10 minutes boiling, followed by 10 minutes steaming at 10 pounds pressure, was employed in sample 84. Both methods were failures as parboiling practices. The kernels were still opaque and up to 46% breakage was obtained during shelling. Better results were obtained with samples 85 and 86 which were soaked for 4 hours, boiled for 5 and 10 minutes, and steamed afterwards for 10 minutes at 15 pounds pressure. A low average breakage of 2.3% resulted from shelling and an average thiamine content of 2.16 μg/g was found in the milled product.

To ascertain whether vacuum application has any beneficial effect on vitamin retention, two samples, numbers 87 and 88 (see *e* Table I), were treated according to the principles of rice conversion. To the first sample, number 7, a vacuum was applied for 30 minutes which was followed by 60 pounds hydraulic pressure at 70°C for 2½ hours. The thiamine content of the milled product amounted to 2.48 μg/g. The second sample, number 88, was treated the same way except that the vacuum application was omitted. The milled end product showed a thiamine content of 2.35 μg/g. Both samples were milled to the same degree of bran removal as all other samples. The small difference of 0.13 μg/g is considered to be insignificant and falls within the experimental error. These results are typical of 15 other experiments carried out by similar procedures.

Thiamine in Soaked Rice. The data of Table III indicate that during soaking of rough brown rice, thiamine penetrates from the outer layers of the kernel into the inner layers, and that soaking has a favorable effect on the thiamine content of the milled rice. Sample 1, without soaking, had a thiamine content of 0.44 μg/g. Sample 2, after 8 hours of soaking, had a thiamine content of 1.03 μg/g. Both samples were milled to the same degree of bran removal. Similar results were obtained in the remaining rough and brown rice samples.

Rough rice soaked in water lost small amounts (up to 4.04%) of its thiamine content in the steep water. These results indicate that only small amounts of thiamine are lost in the steep water in mills where modern commercial practices of parboiling are employed. The losses for brown rice amounted to 10.9% after 8 hours and 16% after 36 hours of soaking, and are of importance where brown rice is soaked for considerable time before it is prepared for human consumption.

Summary

A study has been made of the effect of simple, controlled, standardized methods of parboiling of rough rice on the thiamine content of the milled product (milled to approximately 10% bran removal). These methods included boiling; boiling and steaming; steaming; soaking and steaming; soaking, boiling, and steaming. The data showed that boiling alone is unsatisfactory. Of the other methods tested, the best results were obtained from the following procedures:

Boiling for 20 minutes, followed by 15 minutes steaming at 5 pounds pressure—this method produced fairly well parboiled rice, with a thiamine content of 2.13 μg/g and a breakage of 4%. The average thiamine content of the milled nonparboiled samples was 0.60 μg/g and the average breakage 3.8%.

After 15 hours soaking at room temperature (27°C), followed by 25 minutes steaming at 15 pounds pressure, the thiamine content was 2.71 μg/g and the breakage 6.1%.

After 45 hours soaking at room temperature (27°C) or a minimum of 6 hours soaking at 35°C, followed by 10 minutes steaming at 15 pounds pressure, the thiamine content was 2.20 μg/g and the average breakage 3.1%.

After 6 hours soaking at 40°C, followed by 15 minutes steaming at

15 pounds pressure (Malekized process), the thiamine content was 2.20 μg/g and the breakage 0.9%.

After a minimum of 4 hours soaking at 60°C, a minimum of 2 hours soaking at 70°C, 2 hours soaking at 80°C, at least 30 minutes soaking at 90°C, followed by 10 minutes steaming at 15 pounds pressure, the average thiamine content was 2.30 μg/g and the average breakage 4.2%.

Data are included on the effect of parboiling rough rice on breakage during shelling. It was found that prolonged boiling reduced breakage from an average of 37.8% to 27.6% and additional steaming reduced breakage further to 6.8% in boiling and steaming tests. Lowest breakage was obtained in soaking and steaming tests when soaking was followed by steaming for at least 10 minutes at 15 pounds pressure. Results are also presented on the effect of soaking of rough and brown rice on the thiamine content of the milled form and of losses of thiamine in the soaking water. After 8 hours of soaking brown rice, the thiamine content of the milled form was 1.03% to 0.44% in the milled nonsoaked sample. Losses in the soaking water for brown rice amounted to 10.9% of thiamine after 8 hours, and 16% after 36 hours of soaking. Only 4.04% thiamine was so lost in rough rice after 36 hours of soaking.

Literature Cited

Aykroyd, W. R.
1932 The effect of parboiling and milling on the anti-neuritic vitamin (B_1) and phosphate content of rice. J. Hyg. 32: 184.
Hennessy, D. J., and Cerecedo, L. R.
1939 The determination of free and phosphorylated thiamin by a modified thiochrome assay. J. Am. Chem. Soc. 61: 179–183.
Jack, H. W.
1923 Rice in Malaya. Malayan Agr. J. 35.
Jones, J. W., and Taylor, J. W.
1935 Effect of parboiling rough rice on milling quality. U. S. Dept. Agr. Cir. 340.
Kik, M. C.
1945 Parboiling. Rice J. 48 (12): 6–8.
1945a Effect of milling, processing, washing, cooking and storage on thiamine, riboflavin and niacin in rice. Arkansas Agr. Expt. Sta. Bull. 458.
——, and Williams, R. R.
1945 Nutritional improvement of white rice. Bull. Natl. Research Council 112: 1–76.
Subrahmanyan, V., Sreenivasan, A., and Das Gupta, H. P.
1938 Studies on quality in rice. I. Effect of milling on the chemical composition and commercial qualities of raw and parboiled rices. Indian J. Agr. Sci. 8: 459–486.

STUDIES OF THE COMPOSITION OF THE WHEAT KERNEL. III. DISTRIBUTION OF ASH AND PROTEIN IN CENTRAL AND PERIPHERAL ZONES OF WHOLE KERNELS[1]

V. H. Morris, Thelma L. Alexander, and Elizabeth D. Pascoe[2]

(Received for publication July 12, 1946)

Fundamental knowledge of the relationship between the ash and protein distribution in the wheat kernel and in the mill streams is of marked importance to the flour milling industry. Adequate knowledge regarding this distribution has been difficult to obtain. In the past the principal source of information has been the circumstantial evidence secured through studies of the composition of the different flour and feed grades and the mill streams from which they are combined. Recently the authors (1945) suggested microdissection as a means of obtaining more accurate information on the distribution of ash and protein in the wheat kernel. This was accomplished by securing and analyzing material from definite parts of the kernel. The technique employed and the results obtained through its use were reported in a preliminary study of thè distribution of ash and protein in center sections. In order to correlate the results obtained by dissection with those obtained in milling, it seemed desirable to apply the technique to whole kernels. This paper describes a technique for dissecting whole kernels and gives analytical data obtained in its application to several samples.

Material and Methods

The material consisted of one variety of each of three principal classes of wheat, namely, Thorne (soft red winter), Tenmarq (hard red winter), and Thatcher (hard red spring). The sample of Thorne was obtained from a single plot grown at Wooster, Ohio, in 1943. Composite samples of the other two classes were used. The Tenmarq composite was made up of equal numbers of kernels from individual samples grown at five locations in the central and southern Great Plains in 1944. The composite sample of Thatcher was made up of equal numbers of kernels from individual samples grown at seven locations in the northern Great Plains region in the same year.

The equipment and general technique used in the dissection has been described previously by Morris, Alexander, and Pascoe (1945). In this study the kernels were hydrated by exposure for 24–48 hours in a closed vessel containing an open dish of water and the germ, with its bran covering, was then removed with a scalpel. The germ and degermed kernels were dried for 2 hours at 130°C and weighed. In preparation for the dissection, each degermed kernel was first cut transversely at midsection and then each half kernel was cut longitudinally through the crease. The brush ends were mounted together in pairs on "Lucite" microscope slides by means of a solution of "Lucite" in chloroform. The germ ends also were mounted in the same manner.

---

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Senior Chemist, Junior Botanist, and former Assistant Chemist, respectively, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

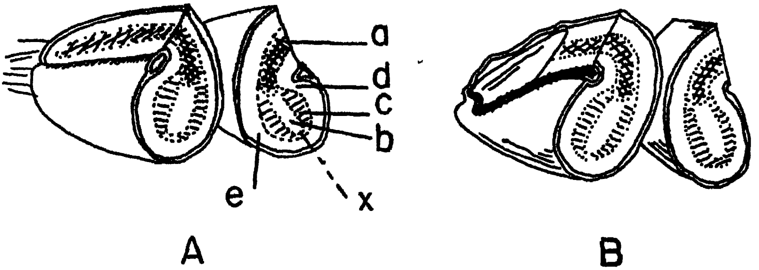


Fig. 1. Portion of kernel included in various endosperm fractions. Central zone: (*a*) "center over crease," (*b*) "center in cheek," (*c*) cheek. Peripheral zone: (*d*) endosperm next to crease, (*e*) outer endosperm, (*x*) line of separation between crease and outer portions of peripheral zone and bran fractions. (*A*) brush half of kernel; (*B*) germ half of kernel.

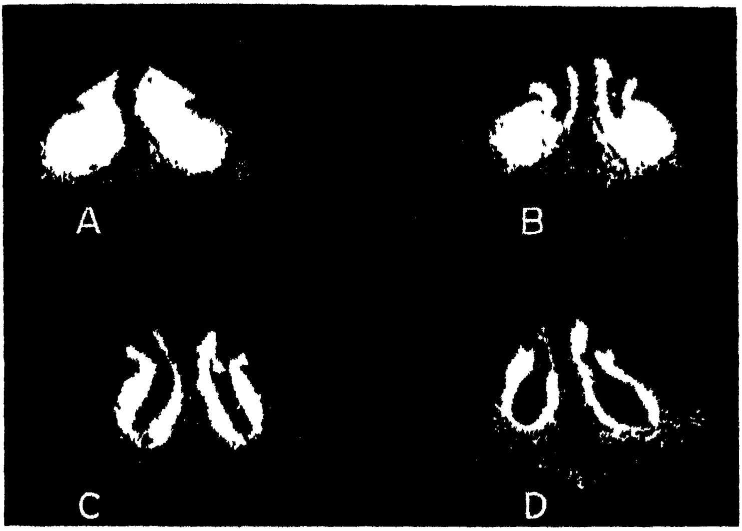


Fig. 2. Successive stages of dissection of brush halves: (*A*) mounted and ready for dissection; (*B*) after removal of center over crease; (*C*) after removal of center in cheek; (*D*) after removal of cheek. (Magnification 6 diameters.)

The endosperm was separated into five fractions. The three fractions removed from the central zone were designated as "center over crease," "center in cheek," and "cheek" endosperm. The remaining peripheral endosperm was divided into two fractions; that next to the crease was called "crease" endosperm, and the endosperm next to the outer part of the kernel was designated as "outer endosperm." The bran was separated into two fractions, "crease bran" and "outer bran." The germ with attached bran constituted another fraction. The portion of the kernel included in each fraction is illustrated by the drawings in Figure 1. The appearance of the kernel during several stages in the dissection is shown in Figure 2.

The "center over crease" and "center in cheek" were secured by drilling, using a No. 79 or 80 wire-gauge twist drill, whereas the "cheek" was obtained by drilling with a No. ½ dental burr. An incision was then made at the base of the cheek dividing the remainder of the kernel into two parts—"crease" and "outer"; the endosperm of each part was separated from the bran by cutting and scraping with a sharp scalpel. The dissected fractions were dried for 1 hour at 135°C, weighed and ground in a micro-Wiley mill. Protein was determined on 6–12 mg subsamples by a micro-Kjeldahl procedure. Ash content was determined by ashing the remainder of each fraction in a semimicro platinum dish, the weighings being made on a microanalytical balance.

Results

The dissection and analytical data are presented in Table I. For convenience in discussion, the quantities of dry matter, ash, and protein in certain fractions have been summed or averaged. The data for the center over crease and center in cheek are referred to as "center endosperm"; these fractions combined with that from the cheeks are referred to as "central zone." The crease and outer endosperm are referred to as "peripheral zone" and the crease and outer bran as "total bran," etc. The data obtained by calculation rather than by analysis are shown in italics in Table I.

One of the difficulties encountered was in obtaining a reasonable recovery of the original undissected kernels. In this study losses of 14, 13, and 22% of dry matter occurred with the soft winter, hard winter, and hard spring varieties, respectively. These undoubtedly represent fine particles lost to the air in drilling as well as losses in the handling of dissected material. Unfortunately there was no way of ascertaining how this loss should be apportioned among the several fractions, but the high proportion of bran and germ shown in Table I indicates that the losses were chiefly in the endosperm fractions.

Ash. In all three varieties the lowest ash concentration was found in one of the two center endosperm fractions. The ash content of these

TABLE I

Composition of Fractions of Wheat Kernels[1]

Parts of kernel	Number of kernels dissected			Kernel composition								
				Dry weight per 100 kernels			Ash in each fraction or part			Protein in each fraction or part		
	Thorne	Tenmarq	Thatcher	Thorne	Tenmarq	Thatcher	Thorne	Tenmarq	Thatcher	Thorne	Tenmarq	Thatcher
				g	g	g	%	%	%	%	%	%
Endosperm												
Center over crease	200	199	180	0.214	0.233	0.193	0.33	0.42	0.38	7.1	9.5	8.3
Center in cheek	200	199	180	.355	.316	.291	.29	.42	.38	6.2	8.5	8.4
Cheek	200	199	195	.320	.244	.266	.32	.47	.44	7.4	9.5	10.2
Crease	75	75	75	.562	.591	.477	.63	.55	.57	11.6	12.6	13.9
Outer	75	75	75	.921	.844	.745	.54	.56	.53	10.5	13.7	15.1
Center				*.569*	*.549*	*.484*	*.31*	*.42*	*.38*	*6.5*	*8.9*	*8.4*
Central zone				*.888*	*.773*	*.750*	*.31*	*.43*	*.40*	*6.9*	*9.0*	*9.0*
Peripheral zone				*1.483*	*1.435*	*1.222*	*.57*	*.56*	*.55*	*10.9*	*13.2*	*14.5*
Total endosperm				*2.371*	*2.208*	*1.972*	*.48*	*.51*	*.49*	*9.4*	*11.7*	*12.5*
Bran												
Crease	75	75	75	.377	.319	.295	5.31	4.46	4.64	12.9	15.1	14.4
Outer	75	75	75	.460	.352	.329	6.72	6.21	6.76	13.1	14.9	14.6
Germ[2]	200	200	200	.149	.109	.119	5.87	5.38	5.35	25.5	26.8	24.3
Total bran and germ				*.986*	*.780*	*.743*	*6.05*	*5.38*	*5.68*	*14.9*	*16.5*	*16.2*
Total dissected kernel				*3.357*	*2.988*	*2.715*	*2.12*	*1.79*	*1.91*	*11.0*	*13.0*	*13.5*
Wheat kernel												
Degermed kernel				3.763	3.322	3.386	1.65	1.40	1.56	10.0	11.5	12.6
Germ[2]				.149	.109	.119	5.87	5.38	5.35	25.5	26.8	24.3
Whole kernel				*3.912*	*3.431*	*3.505*	*1.81*	*1.53*	*1.69*	*10.6*	*12.0*	*13.1*

[1] Data on a 14% moisture basis.
[2] Includes attached bran.

two fractions was not significantly different within the variety for the hard wheat, but in the soft wheat the "center over the crease" was slightly higher than the "center in the cheek." The "cheek" fraction was higher in ash content than the "center in the cheek" for all three varieties, the smallest difference occurring in the soft wheat. While this may be regarded as evidence of an increasing gradient in concentration from the center of the endosperm to the bran coat, the gradient does not appear very steep in this part of the endosperm. The ash content of the two peripheral endosperm fractions—crease and outer—was considerably greater than the central zone fractions for all three varieties. The average ash content of the peripheral zone exceeded that of the central zone by 0.26, 0.13, and 0.15% for Thorne, Tenmarq, and Thatcher respectively. Although only two principal endosperm zones are represented in the dissections, the results corroborate those previously obtained by Morris, Alexander, and Pascoe (1945) in that they indicate the existence of major differences in concentration of ash in various parts of the endosperm. While it will be necessary to analyze a number of samples of the different classes of wheat to establish evidence of fundamental difference between them, the greater spread in concentration between the central and peripheral zones in the Thorne sample would seem to have some significance since in commercial milling it is common practice to obtain lower ash patent flours from soft wheat.

The ash content of the endosperm as a whole (i.e., of the five endosperm fractions) was lowest for Thorne (0.48%), highest for Tenmarq (0.51%), and intermediate for Thatcher (0.49%). These figures all appear somewhat high considering that straight grade flours containing considerably lower ash contents are commonly milled from commercial lots of these wheats. This point is considered further in a later part of the paper.

Protein. The lowest concentrations of protein in all three varieties were found in the center endosperm fractions. The "cheek" fraction exceeded the "center in the cheek," the difference ranging from 1.0 to 1.8% in all three varieties, suggesting the existence of a slightly increasing gradient in protein content as was found also for ash. In general, however, the data indicate that differences in the composition of the central zone within a variety are not marked with respect to protein.

The protein content of the two fractions constituting the peripheral zone was considerably higher than that of the central zone in all three varieties. The increase amounted to 60, 47, and 61% for Thorne, Tenmarq, and Thatcher, respectively.

Comparison of Dissected Fractions and Experimental Mill Streams.

It is a common observation that major differences in ash and protein content such as these found in dissected fractions also occur in flour streams obtained in commercial and experimental milling. The principal difference between the two processes is that in dissection it is possible to obtain endosperm fractions free of bran; whereas, in milling, the flour streams may be contaminated with a small quantity of pulverized bran and germ particles. This is especially true of flour streams from the tail end of the mill. To determine if the range in composition of flour streams obtained by milling agrees reasonably well with that found in pure endosperm obtained by dissection, 2,500 g of the Thorne sample were milled on a Buhler experimental mill. The yield obtained by dissection and through mill streams and the analytical data are presented in Table II.

TABLE II

COMPARATIVE YIELD AND COMPOSITION OF FRACTIONS FROM THORNE SOFT WINTER WHEAT OBTAINED BY DISSECTION AND BY MILLING[1]

Dissection fractions	Dry matter proportion of total dissected[2]	Ash	Protein	Buhler mill streams	Dry matter proportion of total milled	Ash	Protein
		%	%			%	%
Endosperm							
Center over crease	5.5	0.33	7.1	1st break flour	9.4	0.29	7.1
Center in cheek	9.1	.29	6.2				
Cheek	8.2	.32	7.4	2nd break flour	9.8	.34	9.0
Crease	11.4	.63	11.6	3rd break flour	3.6	.60	11.8
Outer	23.5	.51	10.5	1st reduction flour	21.5	.36	9.1
Center	*14.6*	*.31*	*6.5*				
Central zone	*22.7*	*.31*	*6.9*	2nd reduction flour	11.3	.47	9.9
Peripheral zone	*37.9*	*.57*	*10.9*	3rd reduction flour	8.0	.61	11.0
Total endosperm	*60.6*	*.48*	*9.4*	*Total flour*	*63.6*	*.41*	*9.4*
Bran							
Crease	9.6	5.31	12.9	Bran	20.6	5.91	14.3
Outer	11.8	6.72	13.1				
Germ[3]	3.8	5.87	25.5	Shorts	10.8	2.71	13.5
Total bran and germ	*25.2*	*6.05*	*14.9*	*Total bran and shorts*	*31.4*	*4.84*	*14.0*
Total dissected kernel	*85.8*			*Total milled products*	*95.0*		

[1] Data on a 14% moisture basis

[2] Percentage of whole undissected kernel.

[3] Includes attached bran.

The lowest concentration of ash, which was found in the first break flour (0.29%), was similar to that of the lowest ash dissected fraction—"center in the cheek"; in fact it agrees, within the limits of analytical error, with the ash content of the whole central zone (0.31%). The second break and first reduction flours were somewhat higher in ash content than central zone fractions. These two streams together with the first break represented 40.7% extraction with an ash content of 0.34%, compared with 22.7% extraction and 0.31% ash for the central zone obtained by dissection. The high ash flour streams, third break (0.60%) and the third reduction (0.61%), contained about the same quantity of ash as the highest dissected fraction—crease endosperm (0.63%). Similarly, the lowest-protein flour stream (first break, 7.1%) was about equal in concentration to the average of the central zone (6.9%), and the high protein flour streams (third break, 11.8%, third reduction, 11.0%) were about equal in content to the two peripheral endosperm fractions (11.6 and 10.5).

While it is recognized that there is a distinct limit to which experimental milling can be interpreted in terms of the commercial process, the results of this study suggest that the relatively high ash of certain flour streams may be accounted for by the ash content of peripheral endosperm. The results provide little evidence that incorporation of pulverized bran was an important factor in determining the ash content of any of the flour streams. In fact the trend is in the opposite direction since the ash content of the total dissected endosperm of the Thorne sample was 0.48% compared with 0.41% for the total flour obtained by milling. Further evidence of a discrepancy between dissected and milled results was found in the data obtained on Tenmarq and Thatcher. Individual samples of Tenmarq from each of the five locations and of Thatcher from each of the seven locations represented in the respective composites for dissection were milled separately on the Buhler mill and the results for each variety were then averaged. The flour yield, ash, and protein obtained from dissection and from milling of the three varieties, including Thorne, were as shown in Table III.

TABLE III

Dissected Endosperm vs. Mill Stream

Variety	Total dissected endosperm			Straight grade flour		
	Yield	Ash	Protein	Yield	Ash	Protein
Thorne	60.6	0.48	9.4	66.8	0.41	9.4
Tenmarq	64.3	.51	11.7	68.0	.43	11.8
Thatcher	56.3	.49	12.5	62.4	.46	12.2

The difference in ash content between dissected endosperm and straight grade flour is particularly wide with the Thorne and Tenmarq samples, for which the most satisfactory dissection and milling results were obtained. Whether the variations are due to experimental error or to differences inherent in the two processes cannot be determined with certainty with the data at hand. It is possible that the dissected endosperm ash value is relatively too high because of significant losses of material from the lower ash central zone endosperm during the process of dissection. The other possibility is that the shorts produced in the milling operation may carry appreciable quantities of flour containing high ash peripheral endosperm, which would result in lowering the ash content of the straight flour. It may be noted, however, that the discrepancy does not appear in the protein determinations, there being excellent agreement in regard to this constituent between dissected endosperm and straight grade flour in all three varieties.

Summary

A microdissection technique previously developed was employed to separate whole wheat kernels into fractions as follows: (1) five separations by zones of pure endosperm, (2) two of bran, and (3) one of the germ (including attached bran). These were obtained from three classes of wheat—soft red winter (Thorne), hard red winter (Tenmarq), and hard red spring (Thatcher). The Thorne sample came from a single location, whereas the hard wheat samples were composites representing a number of locations all grown in 1944.

In all three varieties the lowest concentration of ash was found in one of the "center" endosperm fractions. Likewise in all three varieties a slight but consistent increase in ash content of the "cheek endosperm" fraction was found as compared with the "center in the cheek." The ash content of the peripheral endosperm fractions was considerably greater than any of the central zone fractions, although the magnitude of the difference varied considerably among the three varieties. In general, similar results were obtained with respect to the distribution of protein in the various endosperm fractions. These facts furnish considerable evidence of an increasing gradient in ash and protein content from the center of the endosperm outward.

A comparison of the ash and protein contents of the dissected fractions with those of Buhler flour mill streams of Thorne soft wheat showed that the lowest-ash dissected fraction (center in cheek, 0.29%) was identical with that of the lowest-ash flour stream (first break, 0.29%). The highest-ash flour streams (third break, 0.60%, and third reduction, 0.61%) were slightly lower than the highest-ash dissected fraction (crease endosperm, 0.63%).

The ash content of total dissected endosperm in all three varieties was somewhat higher than that of the straight grade flours. The protein contents of total dissected endosperm and straight grade flours, however, were in close agreement.

Literature Cited

Morris, V. H., Alexander, T. L., and Pascoe, E. D.
1945 Studies of the composition of the wheat kernel. I. Distribution of ash and protein in center sections. Cereal Chem. 22: 351–361.

PHOTOMICROGRAPHIC STUDIES OF WHEAT STARCH. II. AMYLOLYTIC ENZYMES AND THE AMYLASE INHIBITOR OF THE DEVELOPING WHEAT KERNEL[1]

R. M. Sandstedt and O. C. Beckord

Department of Agricultural Chemistry, Nebraska Agricultural Experiment Station, Lincoln, Nebraska

(Received for publication June 26, 1946)

This paper supplements the one concerning the development of starch granules in wheat (Sandstedt, 1946) with photomicrographs and chemical data concerning amylase activity in the developing wheat kernel.

Relationships between starch deposition and amylase activity in growing plants have not been extensively studied. Until recently methods for quantitatively differentiating the actions of alpha- and beta-amylase were not available. Teller (1938) used photomicrographs of immature starch granules to support his hypothesis that the growth of starch granules was due to the synthetic action of amylases. Bernstein (1943) attempted to relate amylase activity to the rate of starch formation in the developing *maize* kernel. He concluded that the amylases were not active as synthetic agents. Chrzaszcz and Janicki (1936) reported liquefying, dextrinizing, and saccharifying action of enzymes from the grain of wheat, oats, barley, and rye immediately after blossoming and showed that the liquefying and dextrinizing functions gradually disappear, with a partial loss of saccharifying function, as the grain ripens. Sandstedt (1946), while studying the development of starch in wheat, noticed that the starch deposited in the pericarp was digested during the development of the kernel.

Materials and Methods

Kernels of wheat were selected from greenhouse-grown plants at several stages of development. The stages of kernel growth referred to in this paper as *A*, *B*, *C*, etc., correspond to those shown in Figure 1 (reprinted from Sandstedt, 1946). The stage indicated by *G*+ represents about four days later than *G*.

Preparation of Samples. At stage *D* the kernel was sufficiently developed to allow a separation of the embryo, pericarp, and endosperm.[2] Each tissue was washed with water as soon as it had been isolated, since in dissection there was possibility of considerable contamination of one part by the juices from another. Such washing necessarily introduced some error in the quantitative determinations of enzyme action.

---

[1] Published with the approval of the Director as paper No. 396, Journal Series, Nebraska Agricultural Experiment Station.

[2] We are indebted to James Fleming for the dissection and preparation of the samples.

The immature whole kernels and the dissected tissues were dried at room temperature in a current of air from an electric fan. Sufficient material was prepared to give at least 50 mg of dried sample for each analysis.

The endosperm was prepared for analysis by grinding in a mortar. The pericarp and whole kernels were ground with glass. A 50 mg (air-dried) sample was extracted with 5 ml of a 0.2% $CaCl_2$ solution for one hour at 30°C, and then centrifuged.

The dissection of the wheat kernel, especially in the early stages of growth, was tedious and time consuming: at stage *D* a dry endosperm weighed approximately 1 mg. Accordingly, the enzyme methods were modified for the use of small quantities of material.

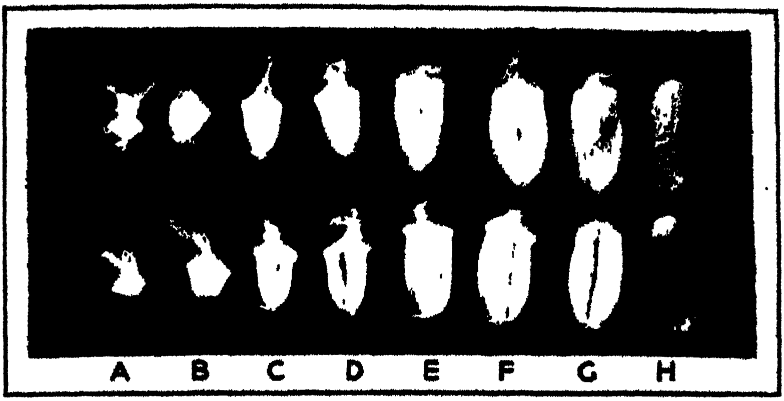


Fig. 1. Wheat kernels showing change in size and form during development (×2.5).
A. Unfertilized ovary.
B-G. Kernels approximately representative of development to be expected at 2-day intervals.
H. Mature dry kernel.

Alpha-Amylase Determination. The method of Sandstedt, Kneen, and Blish (1939) was modified for use of small quantities of material as follows: 4 ml of buffered alpha-amylodextrin substrate was treated with 2 ml of enzyme extract (equivalent to 20 mg of dry tissue). The reaction was carried out in 15 ml tapered centrifuge tubes in the 30°C water bath. One-half ml of iodine-potassium iodide solution ("solution B") was measured into ½ dram vials. Two drops (0.1 ml) of the reaction mixture was added to the 0.5 ml of iodine solution to follow the progress of the reaction.

For the micro amounts of alpha-amylase found in the endosperm, the "overnight method" (Kneen, Sandstedt, and Hollenbeck, 1943) was used wherein 1 ml extract was allowed to act on 4 ml substrate for 18 hours. The reaction was then completed by the addition of 1 ml of an extract of wheat malt of known activity.

The unit of alpha-amylase activity as defined by Sandstedt, Kneen, and Blish (1939) is used in this paper—the amount of alpha-amylase which (under the influence of an excess of beta-amylase) will dextrinize 1 g of boiled soluble starch in one hour at 30°C (usually expressed as units per gram of sample).

Beta-Amylase Determination. The method of Kneen and Sandstedt (1941) was modified for use of small quantities of material as follows: 1 ml of tissue extract was added to 8 ml of 2% starch substrate and 3 ml of water. The reaction was carried out in a 50-ml Erlenmeyer flask at 30°C. At the end of the 15-minute reaction period, 8 ml of 1% H_2SO_4 solution were added. A 5-ml aliquot was taken for ferricyanide reduction. The unit of beta-amylase activity as defined by Kneen and Sandstedt (1941) is used in this paper—the amount of beta-amylase which will convert 1 g of boiled soluble starch to maltose in one hour at 30°C (usually expressed as units per gram of sample).

Photomicrographs. The technique and equipment for taking the photomicrographs was the same as that used by Sandstedt (1946).

Experimental Error. Clean, quantitative separation of tissues was difficult in the early stages of kernel development. The endosperm tended to cling to the pericarp, and washing the dissected tissues entailed inevitable losses. Therefore experimental errors were probably quite large. However, the data do serve their purpose; they give a rough quantitative measure of alpha- and beta-amylase in the tissues and indicate the changes which occur during the development of the kernel.

Results and Discussion

Amylolytic Action in the Immature Wheat Kernel. Teller (1938) hypothesized that starch granule growth was due to amylase action, i.e., that the amylases were active as *synthetic* agents in the tissues in which starch was being deposited. The digestion of the pericarp starch during the growth of the kernel shows that an enzyme system capable of attacking the starch granule is present in this tissue (Sandstedt, 1946).

In preliminary trials, wheat kernels at the several stages of development were autolyzed for 24 to 48 hours (immersed in water, 0.2 *M* acetate buffer at pH 4.7, 1:1,000 Roccal[3] solution, or buffer and Roccal solution), and then crushed and examined. In all tests the pericarp starch had been extensively digested, whereas the endosperm starch showed only slight digestion. However, if the kernels of wheat were crushed before autolysis, the endosperm starch was more rapidly digested than the pericarp starch. Such evidence suggested that the enzymes capable of digesting native starch granules were present and active in the pericarp, perhaps absent in the endosperm, and that immature endosperm starch was more readily attacked by enzymes than was pericarp starch.

---

[3] Roccal is a detergent antiseptic obtained from Winthrop Chemical Company, New York City. It was used in these cases to check against the possibility of the starch digestion being due to bacteria or fungal growth during the extended autolysis. Roccal should not be indiscriminately used because under some conditions it has an effect on enzyme action.

The pericarp (with the nucellus) and the endosperm were dissected from a series of kernels of wheat representing about three- or four-day intervals during the first 18 days of kernel development. Each pericarp and each endosperm was allowed to autolyze (in buffered Roccal solution) for 24 hours, was then crushed, and the amount of starch digestion observed. Considerable starch digestion took place during the pericarp autolysis, whereas no evidence of enzyme action could be seen after endosperm autolysis. Apparently the active enzyme system capable of attacking starch granules was present only in the pericarp.

The above procedure was not satisfactory for photomicrographic illustration since the progressive digestive action on a particular field of starch granules could not be followed. Also, pericarp starch granules are not satisfactory for photomicrographic studies of enzyme action because of their small size and previous exposure to enzyme action. Hence, each endosperm was crushed in a drop of 0.2 *M* acetate buffer (pH 4.7) on a glass slide and then sealed under a cover glass; each pericarp also was treated in this way except that *washed*[4] endosperm starch was added as additional substrate. This procedure permitted photomicrographs to be taken of a satisfactory field of starch granules before, as well as after, intervals of autolysis.

For photomicrographic illustration, the action obtained from the pericarp and endosperm dissected from a kernel harvested the 10th day of development was chosen as typical of the entire period of development. Figure 2 shows endosperm and pericarp starch granules in the dispersion made from the pericarp: *A* at the beginning of the digestion, *B* and *C* after 5 hours and 24 hours at 30°C. The pericarp granules were subject to Brownian movement; accordingly, many of those shown in *A* had moved and cannot be identified in *B*; few can be identified in *C*. It is evident that the pericarp contained an enzyme system capable of attacking immature starch granules. Some of the pericarp granules were quite resistant to the enzyme action, whereas the endosperm starch was completely digested in 24 hours.

---

[4] Isolated endosperms were crushed in water and the starch so obtained was washed by suspension, settling, and decantation. Washed starch was used in order to avoid contaminating the pericarp digest with enzymes and inhibitors from the endosperm.

The pericarp enzyme system was less active in those preparations representing late stages of pericarp development. By the 14th to 16th day of development the pericarp had digested most of its own starch and had lost considerable of its amylolytic activity.

The shrinkage, as distinct from erosion, in the immature endosperm granules during the early stages of enzyme action, i.e., the difference in diameter of the granules before and after a period of digestion, as shown in Figure 1 *A* and *B*, is of interest. Such shrinkage is not evident during the action of enzymes on mature native granules, whereas it is marked during the action of beta-amylase on partially gelatinized granules (unpublished data).

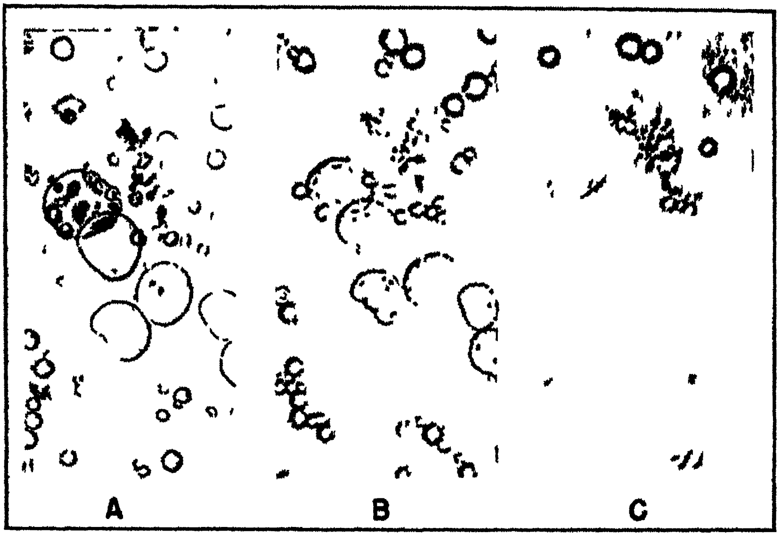


Fig. 2. Action of pericarp enzymes on immature pericarp and endosperm starch granules (×600)
A. Starch at initiation of digestion
B. After 5 hours.
C. After 24 hours.

Figure 3 illustrates the absence of autolytic starch granule degradation in the crushed endosperm during a 72-hour period. If enzymes capable of digesting native starch granules were present in the endosperm, they were apparently in an inactive form. This does not preclude the presence of beta-amylase, which is unable to attack undamaged immature starch granules. (It is shown later in this paper that beta-amylase is present in the endosperm.)

The absence of starch digestion in the crushed endosperm was not due to the use of a pH other than the optimum, since the occurrence of starch granule digestion in the crushed pericarp and its absence in the crushed endosperm were evident, whether the dispersions were at the natural pH of the crushed tissue, buffered at pH 4.7 with acetate, or at pH 6.0 with phosphate.

The Occurrence of an Amylase Inhibitor. During the course of this work it became evident that the resistance of starch granules to amylase action increased with maturity. The preliminary procedure used for studying resistance to amylase action was to crush the entire kernel of wheat in a drop of diluted buffered saliva and to follow the action of the salivary enzyme by microscopic observation. The salivary amylase had a relatively rapid action on the immature starch; the rate of action decreased with increasing maturity of the starch. However, at a stage of development corresponding about to the attainment of full kernel length, salivary enzyme action ceased to take place. After this stage of development, no more starch digestion took place in the presence of saliva than could be accounted for by the action of the pericarp enzymes alone. Yet if washed starch were treated with saliva, normal digestion occurred. It was then found that extracts of wheat flour and even of wheat malt would inhibit salivary amylase action. Apparently an inhibitor of certain types of amylases was developed in the growing wheat kernel at about the time that full length was attained.

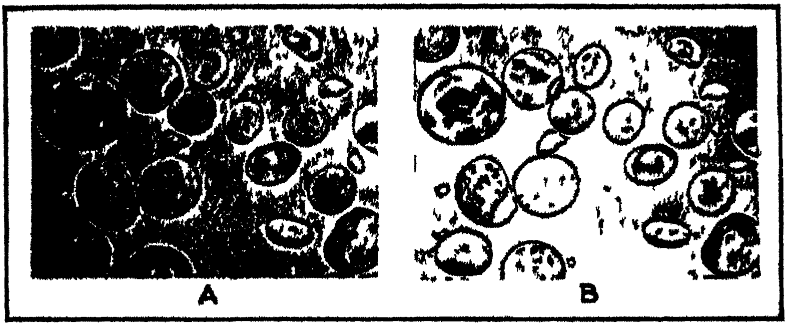


Fig. 3. Inability of the immature crushed endosperm to digest its own starch granules (×600)
A. At the initiation of digestion
B After 72 hours digestion

Since the pericarp and endosperm differ in amylase activity, it was of interest to investigate the possible presence of the amylase inhibitor in each of these tissues. Endosperms and pericarps were dissected from kernels at the full length stage. Each endosperm was crushed in a drop of buffered saliva on a glass slide, sealed beneath a cover glass, and placed at 30°C for digestion. This procedure was repeated with the pericarps, but since at this stage an undried endosperm weighed about five times as much as an undried pericarp, five pericarps were used, together with about 0.5 mg of washed endosperm starch, in each drop of buffered saliva. Check slides were prepared using buffer solutions with no saliva and using 0.5 mg of *washed* endosperm starch [4] alone in buffered saliva.

Typical photomicrographs obtained after a 6-hour digestion are shown in Figure 4. No evidence of enzyme action was shown in the presence of the crushed endosperms (Figure 3 *A*), which indicates an inactivation of the salivary amylase system; as before noted, there was no active enzyme system in the endosperm itself capable of attacking starch granules.

On the other hand, the salivary enzymes were active in the presence of the crushed pericarp (Figure 4 *B*). That most of the action shown in Figure 3 *B* was due to salivary amylase and little due to the pericarp enzyme system is shown by comparison with Figure 4 *C* which shows the action of the enzymes of the crushed pericarp on washed endosperm starch. Apparently a salivary amylase inhibitor was present in the endosperm but not in the pericarp. This is substantiation of the work of Kneen and Sandstedt (1946); they showed that wheat bran extracts caused little inhibition of salivary or bacterial amylases.

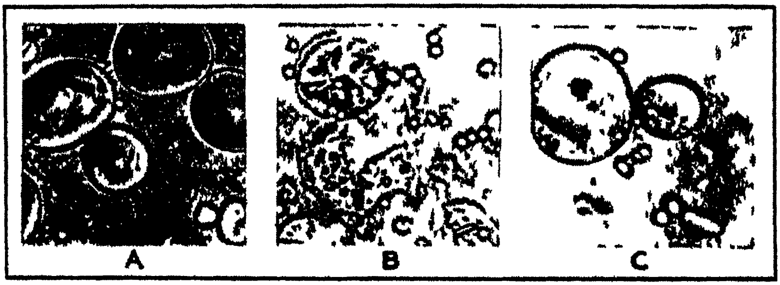


Fig 4 Salivary amylase inhibitor as found in the endosperm and pericarp on the 16th day of kernel development.

All photomicrographs taken after 6 hours digestion (×600)
A Showing complete inhibition of salivary digestion in suspension of crushed endosperm
B Action of salivary and pericarp amylases on washed endosperm starch in the presence of the crushed pericarp—no inhibition of salivary amylase by the crushed pericarp
C Pericarp amylase alone on washed endosperm starch in the presence of crushed pericarp

Correlation of Microscopic Observations with Chemical Analysis. The data of Figure 5 correlating the concentration of alpha-amylase in the kernel and in the kernel tissues with the age of the kernel indicate that the alpha-amylase was practically all in the pericarp with only traces in the endosperm and that the concentration in the pericarp decreased with development.

In accordance with the work reported by Chrzaszcz and Janicki (1936), there was a steady decrease in alpha-amylase concentration in the whole kernel during the growth of the kernel. They, however, could partially restore the original amylolytic power of the grain (but only up to a certain stage of development) by the use of "eleuto-substances" (peptone or weak solutions of salt). They explain the fact that restoration was only partial, i.e., their inability to obtain as high values during the later stages of kernel development as during the early stages, by hypothesizing an excessive accumulation of "sisto-substances" which bind and thus inactivate the amylase to such an extent that the inactivation could not be nullified by "eleuto-substances."

The data of Figure 5 indicate that the decrease in alpha-amylase concentration in the whole kernel during development was due to two factors: 1. A decrease in the content of active alpha-amylase in the pericarp tissue. We were unable to increase the alpha-amylase activity of extracts from tissues of immature kernels by the use of the "eleuto-substances" mentioned by Chrzaszcz and Janicki. 2. The rapid growth of the endosperm (containing only traces of alpha-amylase) with a consequent decrease in the proportion of the kernel occupied by the tissues rich in alpha-amylase.

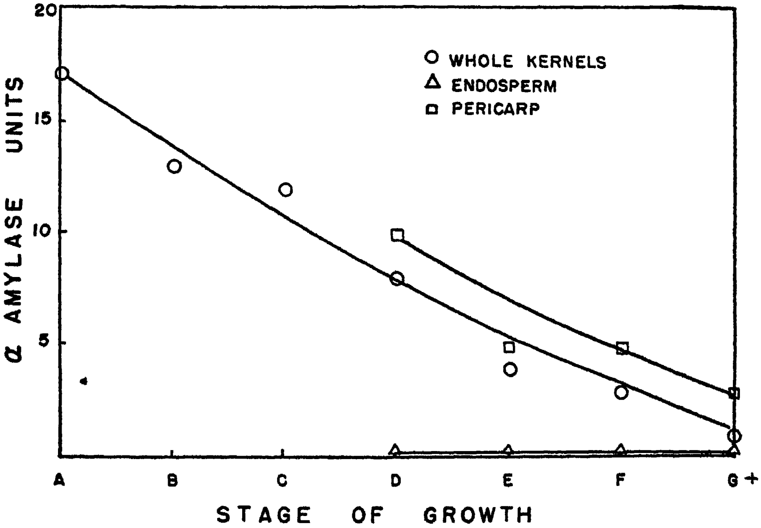


Fig. 5. Relation of stage of wheat kernel development to concentration of alpha-amylase. Alpha-amylase expressed as units per gram of air-dried tissue.

Figure 6, showing the changes in alpha-amylase expressed on a per kernel basis, indicates that there was an increase of alpha-amylase in the kernel during the first week of development (to stage *D*) after which there was a decrease. The increase occurred during the period

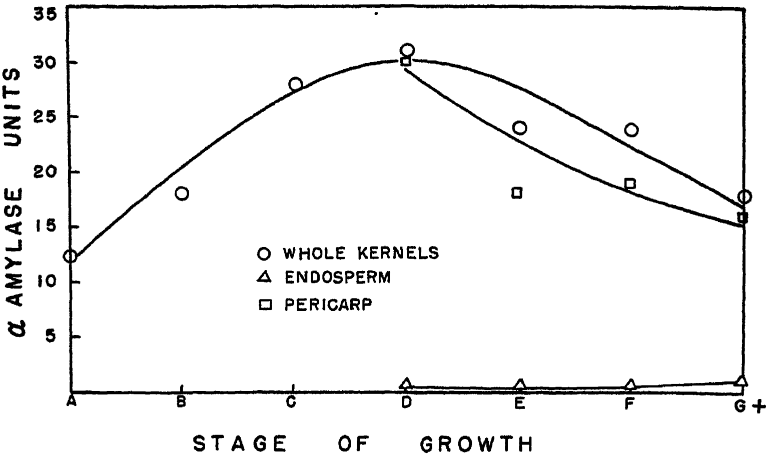


Fig. 6. Relation of stage of kernel development to total alpha-amylase content of the kernel and of the kernel tissues. Alpha-amylase expressed as units per 1,000 kernels.

of rapid growth of the pericarp. The alpha-amylase concentration in the pericarp was decreasing during this period (Figure 5), but the increase in pericarp tissue was rapid enough to cause an increase in total alpha-amylase. After stage *D* the increase in pericarp tissue was too slow to overbalance the decrease in concentration of alpha-amylase.

Figure 7 showing beta-amylase on a per gram basis indicates a

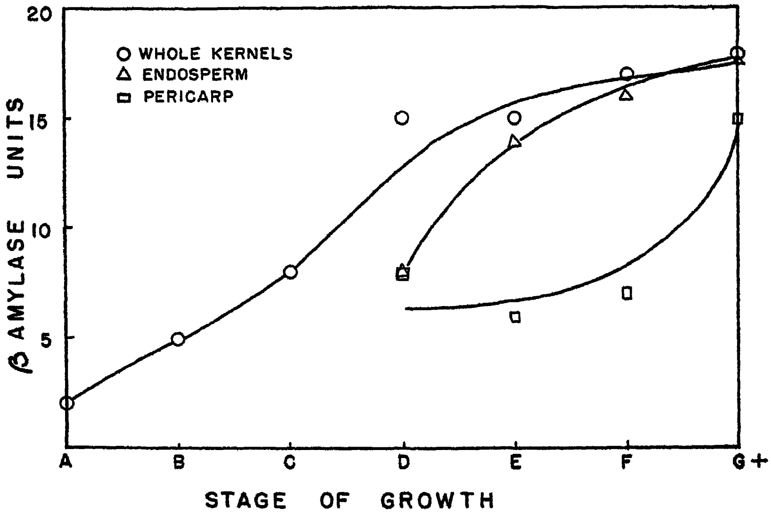


Fig. 7. The relation of stage of kernel development to concentration of beta-amylase. Beta-amylase expressed as units per gram of air-dried tissue.

rapid increase in concentration of beta-amylase in the whole kernel for about the first six days of growth, after which the concentration was almost constant. The concentration of beta-amylase in the endosperm increased rapidly during the period of change in endosperm character, i.e., from a tissue containing little starch to a starchy tissue (stages *D* to *G*). Chrzaszcz and Janicki (1936) noted a decrease in saccharifying power during the later stages of the development of the kernel. This decrease, however, was in saccharification due to the combined action of the alpha- and beta-amylases and so may have been due to the decrease in alpha-amylase (Figure 5). The pericarp also contained considerable amounts of beta-amylase.

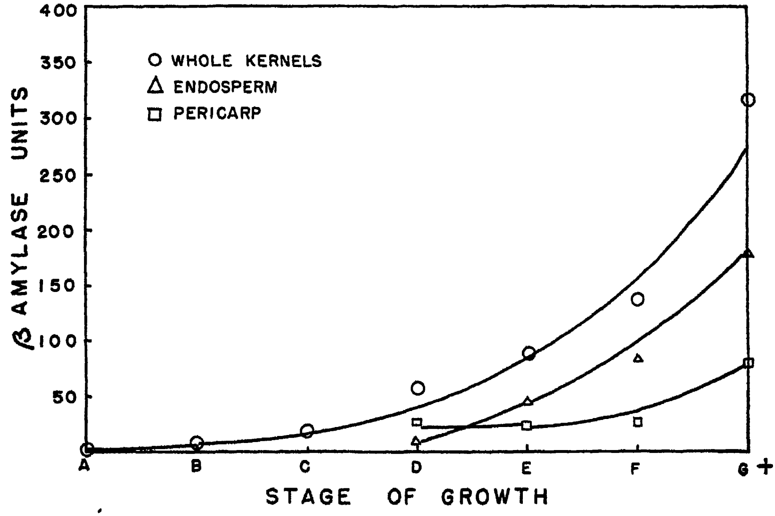


Fig. 8. Relation of stage of kernel development to total beta-amylase content of the kernel and of the kernel tissues. Beta-amylase expressed as units per 1,000 kernels.

Figure 8, showing the units of beta-amylase on a per kernel basis, indicates an increase in the total beta-amylase in the kernel with growth corresponding to the growth of the endosperm.

Embryo. Determinations of amylase activity of the embryo are not reported, as sufficient samples were not obtained to make reliable determinations. Such results as were obtained indicated that beta-amylase was present in smaller amounts in the embryo than in the endosperm and that alpha-amylase was present but in smaller amounts (on a per gram basis) than in the pericarp.

Effect of Proteolytic Enzyme Action on the Extraction of Amylases of the Immature Kernel. Eighteen-hour extractions at 30°C were made on samples of endosperm at stage *G* and whole kernel at stage *D* using a solution containing 0.2% $CaCl_2$ and 10 mg % ficin.[5] No appreciable increase in either alpha- or beta-amylase activity was observed in these extracts, as compared to those made without the use of the proteolytic enzyme. Apparently the decrease in alpha-amylase during the growth of the kernel was not due to a tie-up of the amylase similar to that of beta-amylase in the mature kernel. Chrzaszcz and Janicki (1936) could not increase dextrinizing and liquefying powers by the action of papain.

Summary

Photomicrographs are shown indicating the presence of an amylase system in the pericarp of immature wheat capable of digesting native starch granules. However, no active enzyme capable of attacking native granules could be found in the endosperm at any stage of its development.

Chemical determinations of amylase activity on extracts of pericarp and of endosperm tissue from wheat at several stages during its development substantiated the data obtained by microscopic observation, indicating the presence of alpha-amylase in the pericarp with only traces in the endosperm. The concentration of alpha-amylase decreased during the growth of the kernel, whereas the total alpha-amylase in the kernel increased during the first week of development (period of most rapid growth of pericarp tissue) and then decreased.

The concentration as well as the total quantity of beta-amylase increased during the growth of the kernel, most of the increase being due to the growth of the endosperm.

An amylase inhibitor (which had no effect on the natural wheat or malt amylases but which was effective in inhibiting salivary amylase) appeared in the endosperm at approximately the time that the immature kernel reached full length. This inhibitor was not found in the pericarp tissue.

Literature Cited

Bernstein, L.
1943 Amylases and carbohydrates in developing maize endosperm. Am. J. Botany **30**: 517–526.
Chrzaszcz, T., and Janicki, J.
1936 Amylase during the growth and ripening of grains. Biochem. J. **30**: 1298–1302.
Kneen, E., and Sandstedt, R. M.
1941 Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. **18**: 237–252.
1946 Distribution and general properties of an amylase inhibitor in cereals. Arch. Biochem. **9**: 235–249.
——, ——, and Hollenbeck, C. M.
1943 The differential stability of the malt amylases—separation of the alpha and beta components. Cereal Chem. **20**: 399–423.

---

[5] A proteolytic enzyme of the papain type which is approximately 20 times as active as papain Merck and Company, Inc., Rahway, New Jersey.

Sandstedt, R. M.
1946 Photomicrographic studies of wheat starch. I. Development of the starch granules. Cereal Chem. **23**: 337–359.
——, Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.
Teller, G. L.
1938 Plant diastase in evidence as to the formation and structure of starch granules. Plant Physiol. **13**: 227–240.

SOME OBSERVATIONS ON THE VISUAL COLOR OF TUBULAR AND DISC MACARONI

L. D. Sibbitt and R. H. Harris

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication May 2, 1946)

Fifield, Smith, and Hayes (1937) described a method for producing micro macaroni discs for assessing macaroni-making quality and color characteristics. Cunningham and Anderson (1943) pointed out that difficulty was experienced in obtaining reliable results by the above method and they therefore developed a modified procedure. These workers also studied the influences of variations in processing conditions on the opacity of the discs (Cunningham and Anderson, 1943a, 1943b). Smith, Harris, Jesperson, and Sibbitt (1946), employing equipment similar to that described by Fifield *et al.* (1937), also used a modified procedure of the original technique for making the discs. Smith and associates showed quite clearly the effect of degree and duration of pressing on air bubble size and number, and light transmission of the discs.

Fifield *et al.* (1937) made comparisons of the discs with tubular macaroni. An N-A colorimeter equipped with four color discs was used, but only the yellow component was selected as an index of comparison. Satisfactory results were obtained from five samples representing different durum varieties grown at Langdon, North Dakota, in 1934, but less satisfactory results were secured from four samples from the 1936 crop. There is no direct comparison between the color characteristics of micro macaroni discs and tubular macaroni in the paper by Cunningham and Anderson (1943a).

The study reported in this paper was conducted to obtain further information regarding the effect of degree and duration of pressure on visual color score of macaroni discs, and to compare directly the color scores of disc and tubular macaroni processed from the same series of semolinas.

Materials and Methods

Purified semolinas used in this investigation were experimentally milled from six varieties of durum wheat grown at Langdon in 1943. Of these, Mindum, Carleton, Stewart, Kubanka, and Monad are grown commercially, while L.D. 153 is still in the experimental stage. These varieties were selected because they represent a wide range in macaroni color.

The wheats were milled in an Allis-Chalmers experimental mill and purified in a small scale purifier, and the tube macaroni was processed from the purified semolina using the methods and technique outlined by Harris and Sibbitt (1942). Micro macaroni discs were also made from the purified semolinas at three pressing times and four pressures as discussed by Smith *et al.* (1946). Since these workers also described details of processing the discs, and making light transmission readings, experimental technique will not be outlined in the present paper. The macaroni and the micro macaroni discs were judged visually against a color standard. The color scale ranged from 0 to 10 in increments of 0.5.

Results

Table I shows the individual visual color scores and the means of the micro macaroni discs for the six varieties at three pressing times and four pressures; also the visual color scores for the tubular macaroni. In processing the discs, 2,000 pounds pressure per square inch for 60 seconds produced discs of satisfactory colors with a fairly good range between the varieties, and was selected as the standard for comparison with the macaroni. This choice is also substantiated by the light transmission readings for the discs at the various times and pressures, presented graphically in the paper by Smith *et al.* (1946). This method has the advantage of being comparatively rapid, and the pressure is very convenient to employ.

An extreme variation in color score is evident among the different treatments, and emphasizes the importance of time and degree of pressure in processing disc macaroni. The poor color score of the discs produced at relatively low pressures illustrates the observation of macaroni manufacturers that the first portions of macaroni extruded from the press, before the optimum pressure for the development of satisfactory color is built up, are dull and have poor color. Smith and associates found that the first sections of tube macaroni from an experimental press contained numerous small air bubbles, and attributed the unsatisfactory color characteristics of the product to this fact. Numerous small bubbles interfere more with the passage of light through the material than a few large bubbles.

TABLE I

VISUAL COLOR SCORE OF MACARONI DISCS
(Durum wheats grown in 1943)

Time	Pressure	Carleton	Mindum	Stewart	L.D. 153	Ku-banka[1] 314	Monad[1]	Mean
Sec.	*lb./sq. in.*							
20	1,250	3.0	3.0	3.0	3.0	2.0 D	1.5 DB	2.6
20	1,500	4.0	4.0	4.0	4.0	3.0 D	2.0 DB	3.5
20	2,000	6.0	6.0	6.0	6.0	6.0 D	4.0 DB	5.7
20	3,000	9.0	8.0	8.0	8.0	6.5 D	5.0 DB	7.4
Mean								4.8
60	1,250	4.0	4.0	5.0	5.0	3.0 D	2.5 DB	3.9
60	1,500	7.0	7.0	6.0	6.0	6.0 D	5.5 DB	6.3
60	**2,000**	**9.0**	**9.0**	**9.0**	**8.0**	**7.0 D**	**6.0 DB**	8.0
60	3,000	9.0	9.0	9.0	9.0	7.0 D	6.0 DB	8.2
Mean								6.6
240	1,250	8.5	8.0	7.0	7.0	6.5 D	5.0 DB	7.0
240	1,500	9.0	9.0	8.0	8.0	7.0 D	6.0 DB	7.8
240	2,000	9.0	9.0	8.5	9.0	7.0 D	6.0 DB	8.1
240	3,000	9.0	9.0	9.0	9.0	7.5 D	6.0 DB	8.2
Mean		7.2	7.1	6.9	6.8	5.7	4.6	7.8
Color score of tube macaroni		9.5	7.5	8.5	9.5	7.0	3.0	

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance
Between varieties	5	12.39[2]
Between pressures	3	42.05[2]
Between times	2	54.68[2]
Interactions:		
(Varieties × pressures)	15	0.22
(Varieties × times)	10	0.21
(Pressures × times)	6	5.84[2]
(Varieties × pressures × times)	30	0.21
Total	71	

[1] D = dull, DB - dull brown.
Note: Bold type indicates data selected for comparison with tube macaroni.
[2] Denotes significance at 1% point.

An analysis of variance for the visual color score of the micro macaroni discs at the various pressing times and pressures is given in Table I. Very significant differences are shown for varieties, pressures, and times. Varieties have the least effect, with times the greatest. The data are shown graphically in Figure 1. Pressing for 20 seconds apparently cannot develop the best color irrespective of the pressure applied. Pressing for 60 seconds, however, will yield optimum color provided the pressure is above a minimum, approximately 2,000 pounds per square inch in the present instance. A pressing time of 240 seconds attains maximum color score slightly above 1,500 pounds. The relation between pressure and color score is curvilinear at low pressures for all press times. These results agree rather well with the conclusions of Smith *et al.* (1946), who found similar trends in the effect of duration and intensity of pressure on bubble size and number, and light transmission. The pressure effects on color score are not as sharply defined as they are on bubble properties and light transmission determinations, and this result would be anticipated *a priori* because the latter measurements are quantitative and discrete in character. A correlation coefficient of $+0.743 (N = 78)$ was found between the light transmission readings and the visual color scores for the micro discs. Although this value is not sufficiently high to allow the accurate prediction of color score of disc macaroni from light transmission readings of the discs, it does show a distinct positive relation between the two series of observations. The lack of better agreement apparently lies in the high pigment content of Monad, which is not reflected in the light transmission values, and in the failure of the visual score to differentiate well among the higher pressures.

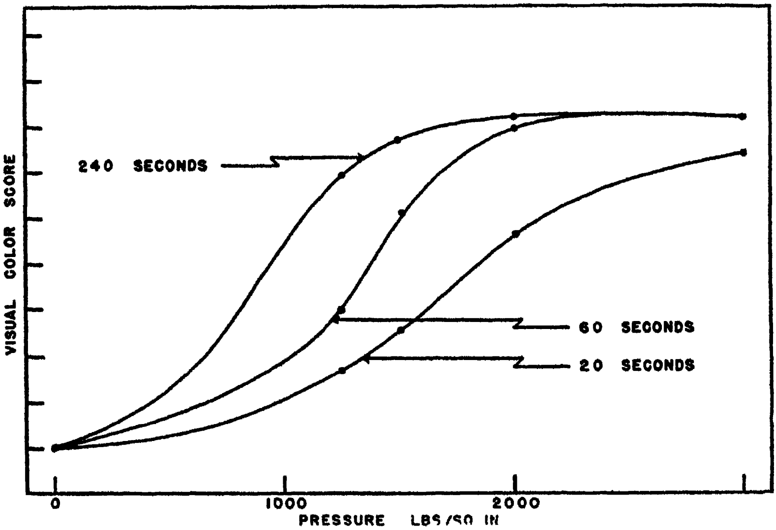


Fig 1. Effect of pressure and pressing time on the visual color score of macaroni discs The curves represent the mean values for three varieties

Comparative macaroni disc color score data representing six durum varieties are shown in Figure 2. The solid bars represent the mean color scores for macaroni from wheats grown at Langdon for six consecutive years (1940–1945). The finer cross-hatched bars show the results obtained on the tubular macaroni for one year only (1943), and the coarse cross-hatched bars denote the data for the discs processed from the same semolina using 60 seconds pressing time and 2,000 pounds pressure. These data yield a direct comparison of the color scores of tubular macaroni obtained from six years' data with the results secured from one year's data on the same varieties. As would be expected, some differences in the actual scores are observed, but the general ranking of the varieties is essentially the same. An indication is also given of the relative grading of the two products within one year. In view of the satisfactory relationship in macaroni color between data from six crops and the results from the 1943 crop, and between the tubular and disc macaroni series for 1943, it would appear reasonable to assume that a similar relationship would exist between tube macaroni and macaroni discs for six years' data. Actually to prove this hypothesis it would be necessary to carry on this study for a number of years. However, with the recent development of a newer and possibly a better method for evaluating small samples of semolina (Martin, Irvine, and Anderson, 1946), the utility of the disc procedure will in all probability be greatly curtailed, especially for nursery samples. It is interesting to note the Mindum and Monad had a higher color score for the micro macaroni disc. In this disc, Monad appeared more translucent than in the tubular macaroni; however, it still possessed the distinct undesirable brown color which is characteristic of this variety. Apparently there is a highly significant positive association between the color scores for the two series of products, but the

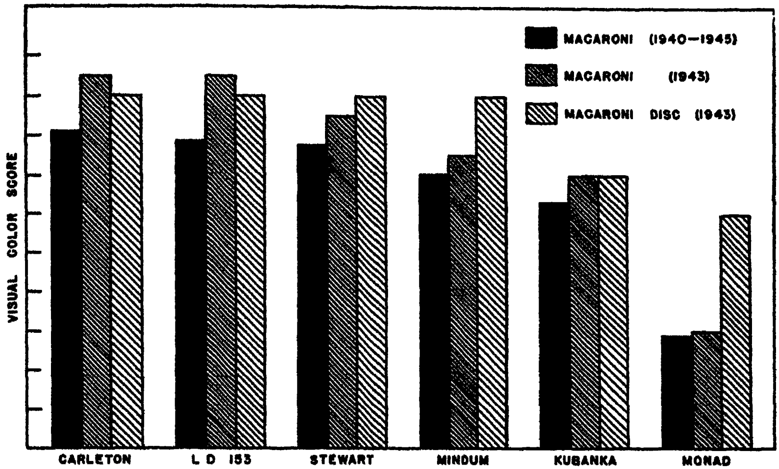


Fig. 2. Visual color scores of tubular macaroni and micro macaroni discs (pressure 2,000 pounds per square inch, pressing time 60 seconds)

number of readings (six) was too small to justify the calculation of a correlation coefficient for subjective data of this nature. It does show, however, that macaroni discs give a good indication of the color ranking of tube macaroni processed from the same material.

Summary

Semolinas were experimentally milled from six durum varieties grown at Langdon in 1943. Tubular macaroni and micro macaroni discs were processed. Three times and four pressures were used in producing the discs.

Very significant differences in the visual color scores of the discs were found between varieties, pressures, and times, with the effect of variety being least marked. A pressing time of 60 seconds and a pressure of 2,000 pounds per square inch gave the most satisfactory results. A satisfactory relationship was evident between the visual color scores of these discs and tubular macaroni made from the same semolina.

A correlation coefficient of +0.743 was found between the light transmission readings and the visual color scores for the micro discs. This value is not high enough to permit the estimation of color score from light transmission readings, owing chiefly to the relatively high pigment content of one of the durum varieties, and to the inability of the visual score to differentiate effectively among the higher pressure treatments.

It is concluded from this study that colors judged from micro macaroni discs could with limitations be used as an index of the color of the actual macaroni.

Acknowledgment

The authors wish to acknowledge the cooperation of Glenn S. Smith in supplying the discs for the visual color examination.

Literature Cited

Anderson, J. A., and Cunningham, R. L.
1943 Micro tests of alimentary pastes. III. The differential response of varieties to processing methods. Can. J. Research C21: 265–275.
Cunningham, R. L., and Anderson, J. A.
1943 Micro tests of alimentary pastes. I. Apparatus and method. Cereal Chem. 20: 171–185.
1943a Micro tests of alimentary pastes. II. Effects of processing conditions on paste properties. Cereal Chem. 20: 482-506.
Fifield, C. C.
1934 Experimental equipment for the manufacture of alimentary pastes. Cereal Chem. 11: 330–334.
——, Smith, G. S., and Hayes, J. F.
1937 Quality of durum wheats and a method for testing small samples. Cereal Chem. 14: 661–673.

Harris, R. H., and Sibbitt, L. D.
1942 Experimental durum milling and processing equipment, with further quality studies on North Dakota durum wheats. Cereal Chem. 19: 388–402.
Martin, V. G., Irvine, G. N., and Anderson, J. A.
1946 A micro method for making macaroni. 23: 568–579.
Smith, G. S., Harris, R. H., Jesperson, E., and Sibbitt, L. D.
1946 The effect of pressure on macaroni discs: Size and number of air bubbles in relation to light transmission. 23: 471–483.

NOTE ON A SEMIMICRO MILLING PROCEDURE FOR DURUM WHEAT[1]

M. H. Fisher and W. O. S. Meredith

Grain Research Laboratory, Board of Grain Commissioners for Canada,
Winnipeg, Manitoba

(Received for publication August 5, 1946)

The semimicro milling procedure described in this note was developed in the laboratory to provide sufficient semolina for quality tests on early generations of durum wheats produced by plant breeders. Its particular advantages, as compared, for instance, with the methods of Fifield, Smith, and Hayes (1937) and of Harris and Sibbitt (1942), are that it can be used with as little as 300 g of wheat and produces a higher yield of semolina. There is a slight sacrifice in coarseness of the semolina, but this is about the same in granulation as, and equal in dress to, a commercial third sizing, and has proved entirely satisfactory for all laboratory tests of macaroni-making quality. Although the flow was designed primarily for small samples, it is now used in this laboratory for large samples as well.

A minimum of 300 g of clean wheat (14% moisture), plus an additional 1% for scouring loss, is recommended for the semimicro procedure; less wheat produces too small an amount of semolina for efficient purification. The moisture content of the wheat is raised to 14.5% with distilled water and, after mixing thoroughly, the wheat is set aside in a closed container to temper for 18 hours. It is then scoured and the weight reduced to 302 g, which corresponds to 300 g at 14% moisture. Just before milling, additional water is added to bring the moisture content of the wheat to 17%.

The flow is shown in Figure 1, and requires four breaks, two reductions, and four purifications. It was designed for an Allis-Chalmers mill with first and second break rolls mounted two-high in the same housing. The wheat thus passes from the first to the second break

---

[1] Paper No. 84 of the Grain Research Laboratory and No. 248 of the Associate Committee on Grain Research (Canada).

without intermediate sifting. For a mill with separate stands for first and second breaks, the sifter may be by-passed after the first break which releases very little semolina. The flow may be modified by changing the first two breaks to suit the available stands of break rolls.

The recommended corrugations and spacing of the rolls are as follows: first break, 18 corrugations, .056 inch; second break, 20 corrugations, .020 inch; third break, 24 corrugations, .008 inch; fourth break, 24 corrugations, .004 inch; first and second reduction, lightly frosted rolls barely touching. All purifications are made with maximum air flow in the machine described by Binnington and Geddes (1936). Other details of the flow are shown in Figure 1.

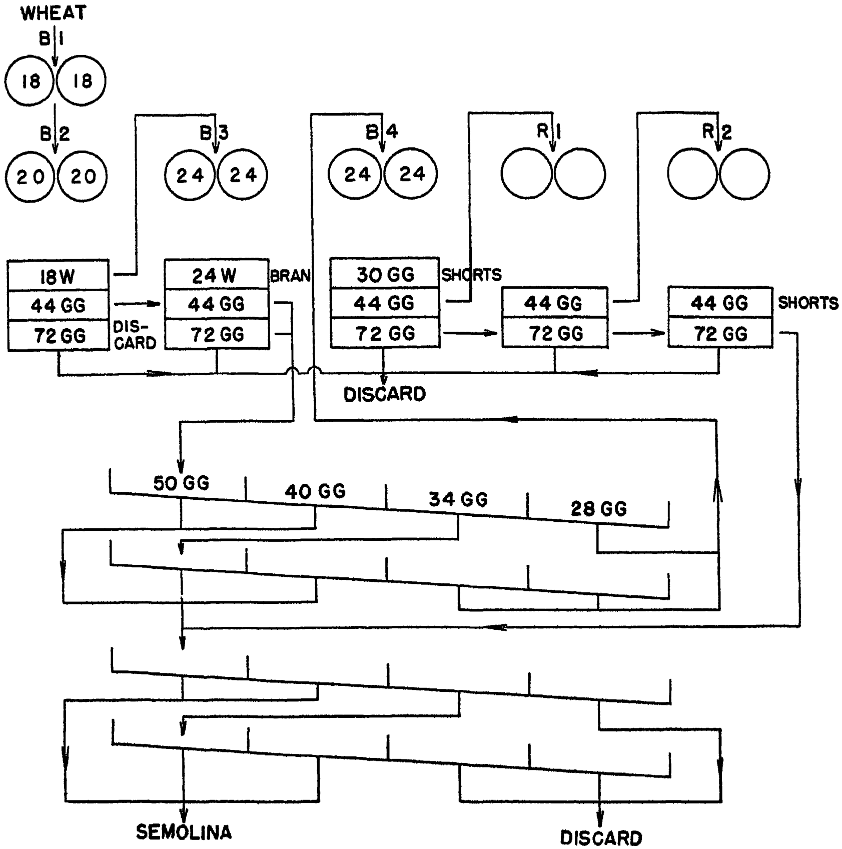


Fig. 1. Flow sheet for milling of wheat and purification of semolina.

To obtain information on the comparative yields and quality of semimicro- and macro-milled semolina, 42 varieties of amber durum wheat were milled by both procedures. Each wheat was a composite sample of the variety grown at several stations in western Canada in different years: 13 varieties in 1941, 16 varieties in 1942, and 13 varieties in 1943. The methods of milling were identical except that 300 g wheat was used for the semimicro procedure and 2,500 g wheat for the macro procedure. Quality was assessed by the disc procedure, described by Cunningham and Anderson (1943), with a dough mixing time of 40 seconds and absorption of 30%. The mean data, for each year and all years combined, for semolina and disc properties are reported in Table I.

TABLE I

MEAN DATA FOR SEMOLINA AND DISC PROPERTIES

Year	Method	Semolina			Discs		
		Yield	Protein	Pigment	Opacity	Yellow[1]	Pigment
		%	%	p.p.m.		%	p.p.m.
1941	Semimicro	51.6	—	4.16	2.67	48.0	3.24
	Macro	51.5	—	4.19	2.72	49.1	3.25
1942	Semimicro	54.2	12.1	3.25	3.71	41.5	2.54
	Macro	56.1	12.1	3.36	3.41	45.8	2.60
1943	Semimicro	52.2	13.1	5.15	2.72	53.8	3.43
	Macro	52.2	13.1	4.86	2.50	52.9	3.69
1941–1943	Semimicro	52.7	12.6	4.19	3.03	47.8	3.07
	Macro	53.3	12.6	4.14	2.88	49.3	3.18

[1] Reflected light.

The mean values for the 42 samples (last two lines in table) show that semolina yields and pigment contents were essentially the same for both procedures and that protein contents were identical. For the discs, opacity and pigment contents were much the same, but percent yellow was slightly higher for the macro milling. Agreement between the mean values for different years was good for 1941 and 1943 and not quite so good for 1942, particularly in semolina yield and percent yellow. The differences are not unduly large and are considered satisfactory. Because of low protein content, durum wheat of the 1942 crop offered more difficulties in milling and in paste preparation than usual, and the divergence between the mean values found for 1942 was not unexpected.

Although all the factors listed in the table are considered when selecting promising varieties, the visual rating of the discs for color is the most useful single index used. Comparison of varieties is always made with Mindum, the recognized standard variety for amber durum wheat, and only varieties that are "equal in quality" to Mindum are considered for licensing and distribution. In all three sets of samples, Mindum ranked high in visual appearance of the discs, and there was good agreement between the semimicro- and macro-milled samples on the varieties that were classed as "equal to or better than" Mindum. Some discrepancies were found among the placings of varieties classed as "inferior to" Mindum but, as such samples are generally not recommended for further testing, their actual rank-order placing is of secondary importance.

This study indicates that the semimicro milling procedure is a most useful one; it meets the requirements of the plant breeder and enables the laboratory to provide information on durum wheat quality at a much earlier stage in the breeding program than would be possible otherwise.

Literature Cited

Binnington, D. S., and Geddes, W. F.
1936 Experimental durum milling and macaroni making technique. Cereal Chem. 13: 497–521.
Cunningham, R. L., and Anderson, J. A.
1943 Micro tests of alimentary pastes. I. Apparatus and method. Cereal Chem. 20: 171–185.
Fifield, C. C., Smith, G. S., and Hayes, J. F.
1937 Quality in durum wheats and a method for testing small samples. Cereal Chem. 14: 661–673.
Harris, R. H., and Sibbitt, L. D.
1942 Experimental durum milling and processing equipment, with further quality studies on North Dakota wheats. Cereal Chem. 19: 388–402.

A MICRO METHOD FOR MAKING MACARONI [1]

V. G. Martin, G. N. Irvine, and J. Ansel Anderson

Grain Research Laboratory, Board of Grain Commissioners for Canada,
Winnipeg, Manitoba

(Presented at the Annual Meeting, May 1946; received for publication July 25, 1946)

The first micro method for evaluating the macaroni-making qualities of durum wheats was reported in 1937 by Fifield, Smith, and Hayes, who prepared discs from 30 g of semolina. Since that time, Cunningham and Anderson (1943) have modified the method somewhat and have introduced an optical opacity measurement as a supplementary means of evaluating quality. Further work with a modified form of the apparatus used by Fifield *et al.* has been recently reported by Smith, Harris, Jesperson, and Sibbitt (1946). While the disc method has been of considerable value in working with small samples of durum wheats, it has been shown by previous investigators that the correlation betwcn the color scores for discs and for macaroni made from the same semolina are not entirely satisfactory. To eliminate the difficulties of working with the disc method while retaining its micro proportions, equipment and method have been developed for producing macaroni from 50 g of semolina. Some of the apparatus used is the same as used in the disc method, some has been modified, and some has been newly designed. In developing the apparatus and method, an attempt has been made to adhere as closely as possible to the routine of commercial practice.

---

[1] Paper No. 85 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, and Paper No. 246 of the Associate Committee on Grain Research (Canada).

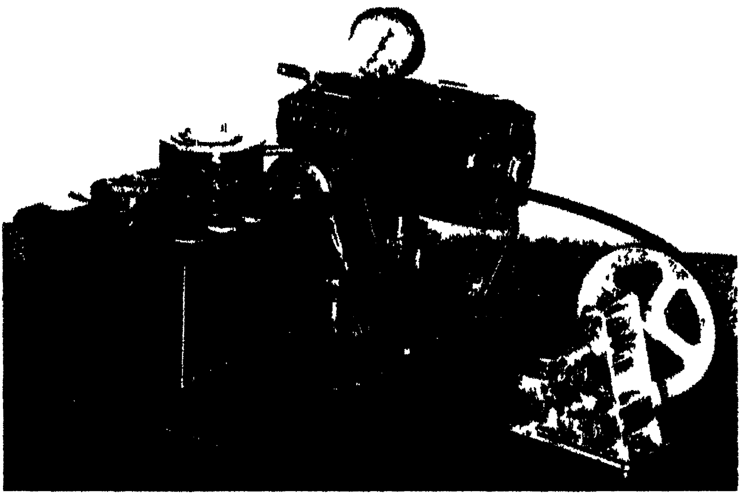


Fig. 1. Mixing unit and belt-driven kneader.

Apparatus

The apparatus can be conveniently described in relation to the five stages of macaroni-making: mixing, kneading, resting in the press, extrusion, and drying.

Mixing Unit. Figure 1 shows the mixing unit. This is essentially the same as that described by Cunningham and Anderson (1943) except for a new mixing spindle and the addition of a vacuum cover over the mixer. The vacuum cover and vacuum gauge are of no concern in this paper; they were added to facilitate experimental work on mixing.

The mixer is directly below the round vacuum cover shown in the photograph. It consists of a small U-shaped mixing trough, 2½ inches square by 2½ inches deep, set in a constant temperature bath, which forms the bulk of the mixing unit shown in the photograph. The trough contains a horizontal spindle with four mixing paddles, each one inch long and set at right angles to each other; two work flush with the ends of the trough and the other two are spaced equally between the ends. The mixing spindle is driven by a reversible motor at 57 r.p.m., and the direction of mixing may be changed by means of the reversing switch. Lids of six brass semolina-conditioning cylinders immersed in the bath, the stirrer motor, and the thermoregulator are also shown in the photograph.

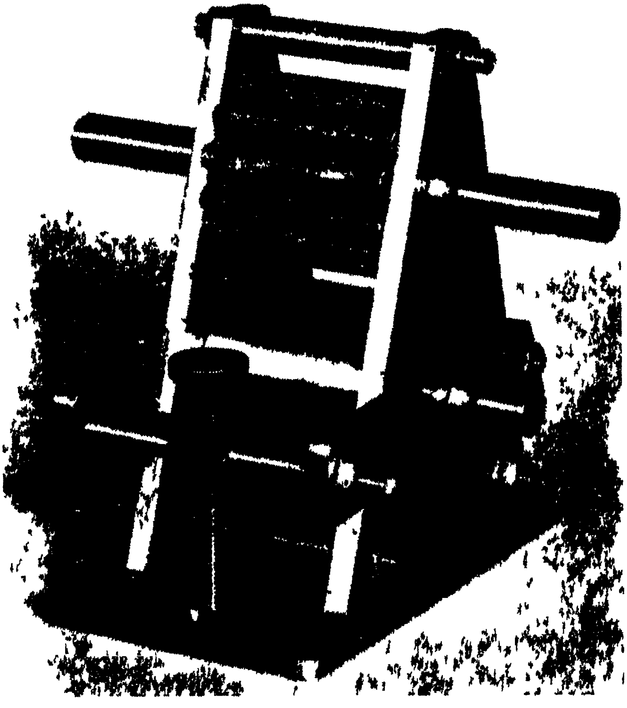


Fig. 2. Belt-driven kneader.

Kneader. Figure 2 is a photograph of the kneader. It is made up of two bronze rolls, 2 inches long by 2 inches in diameter, set in a brass framework 5½ inches high. The upper roll is corrugated with 12 blunt teeth ¼ inch deep, and is driven by a belt and pulley from the motor that drives the mixer; the roll rotates at 25 r.p.m. The lower roll is smooth and rotates freely; its distance from the driving roll can

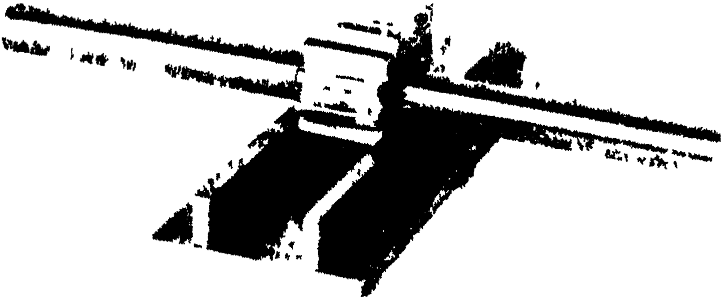


Fig 3. Hand-operated kneading trough

be varied by means of the screw adjustment and lever arms in the foreground of the photograph. The space between the rolls can be varied from ½ inch to zero.

Some work has been done with the hand-kneader shown in Figure 3. The trough consists of two brass strips 9 inches long, ¾ inch wide, and ¼ inch thick, screwed onto a brass plate 9 inches × 3 inches × ¼

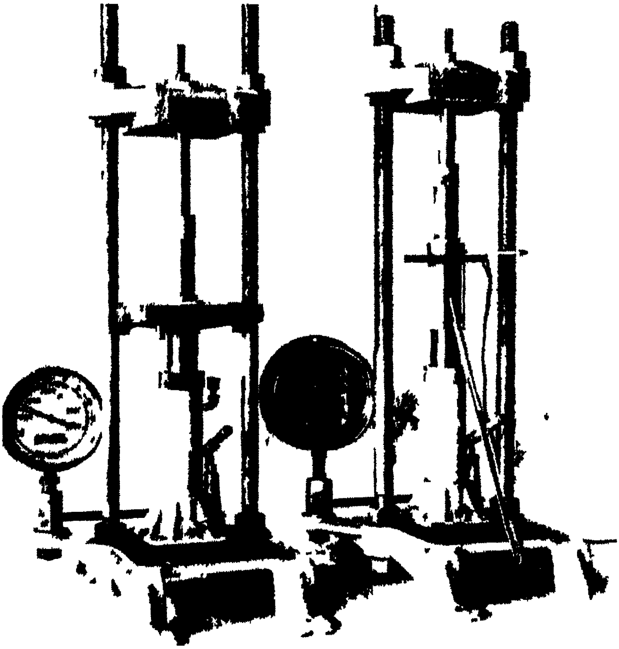


Fig. 4. Rest press, and extrusion press showing macaroni strand emerging from die.

inch; the hand roller is of the same general design as the driven roller described above and is 2 inches in diameter and 1⅜ inches wide. The handles are of solid steel ¾ inch in diameter and 4¾ inches long.

Rest Press. A photograph of the rest press, as it appears in operation, is shown on the left-hand side of Figure 4. The press is a standard Carver laboratory press fitted with a 6,000 pound gauge. Plunger, dough holding cylinder, and end cap, which comprise the additional equipment required, are shown assembled in the photograph and separated in the detail drawing at the left in Figure 5. The plunger is 1 inch outside diameter at the end and is relieved ⅝ inch from the end to facilitate removal from the dough cylinder. A tolerance of 3/1,000 inch is allowed between the cylinder and the end of the plunger. The cylinder is knurled and the end cap milled to permit easy removal of these parts from the assembly.

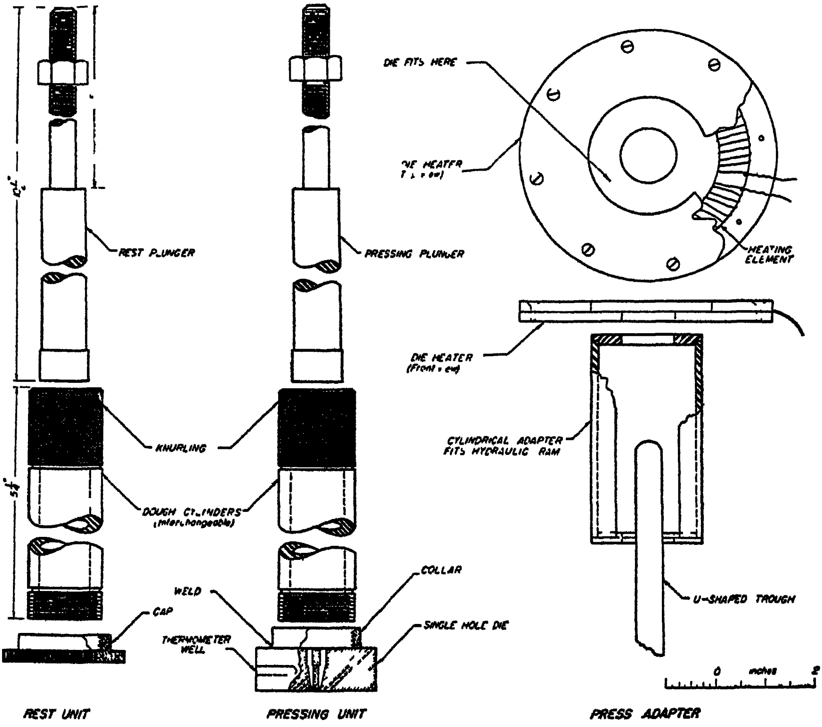


Fig. 5. Detail of rest press and extrusion press equipment.

Extrusion Equipment. The right-hand side of the photograph in Figure 4 shows the extrusion equipment as it appears in operation. This press is identical with the rest press except that it is fitted with a

2,000 pound gauge. Drawings of the extrusion equipment are shown in detail at the center and right-hand side of Figure 5. The plunger and dough cylinder are identical and interchangeable with those of the test press apparatus In addition there are shown the die heater, the single-holed die with threaded collar, and the adapter cylinder fitted with a brass trough. The die was made by F. Maldari and Bros. of New York and is $2\frac{5}{8}$ inches in diameter and $\frac{7}{8}$ inch thick. A bronze threaded collar is welded to the die (with easy-flow silver solder) so that the dough cylinder can be fastened securely to the die. The die heater is made up of a nichrome resistance wire element sandwiched between two discs of tempered masonite, and its temperature is controlled by varying the input voltage by means of a Variac. A trough is necessary to guide the strand of macaroni as it comes from the die and also to prevent it becoming curled and uneven.

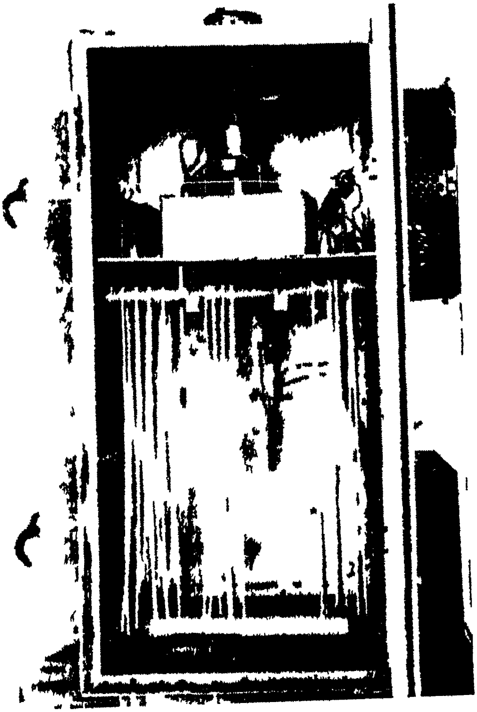


Fig 6 Experimental small scale drying cabinet

Drying Cabinet. Figure 6 is a photograph of the experimental drying cabinet; only a few strands of macaroni are shown in it in order that the controls may be seen. The cabinet is made of tempered masonite on an angle iron frame, 28 inches high, 21 inches deep, and 14 inches wide. It is divided into two compartments by a shelf slotted with holes to allow free circulation of air from the top to the bottom. The upper compartment contains a small variable-speed fan, a Bahnson humidifier, and two 60 watt 12 inch Lumiline Mazda lamps used as heaters. A third lamp of 40 watts is placed across the bottom of the cabinet. The lower compartment contains racks to hold the wooden drying sticks, an Aminco thermoregulator, and the trough for the wet bulb controller. A junction box is mounted on the side of the cabinet and contains the relays for the humidity controller and the thermoregulator, the rheostat for controlling the fan speed, and switches for all the electrical circuits.

Uniform circulation of air is maintained by the fan. An adjustable inlet vent is provided directly above the fan, and there are two outlet vents, 1 inch in diameter, at the bottom of each of the two sides, about 4 inches from the corners. The humidity is controlled by means of a wet bulb recorder of the type described by Binnington and Geddes (1934). In essence it consists of a recording wet bulb thermometer in which the recording chart is replaced by a metal disc cut to a pattern. When the pen makes an electrical contact with the disc the humidifier is turned on; in this way it is possible to obtain any desired humidity-time gradient.

Method

The method presented below is based on the results of an investigation of the various processing factors; while it produces an excellent sample of macaroni, it is subject to change as and if additional study shows the need.

A 50-g sample of semolina is mixed at 31% absorption (14% moisture basis) for 75 seconds, with the water bath at 86°F. The dough is removed from the mixing trough and run through the kneading rolls 10 times. The rolls are set $\frac{1}{16}$ inch apart, and after each run the dough is folded, alternately crosswise and lengthwise. (If the hand kneader is used, 2½ minutes is sufficient to produce a workable dough.) It is then placed in one of the press cylinders, the bronze end cap is screwed on, and the cylinder is placed in the rest press under a pressure of 1,000 pounds. During the 9 minutes it is allowed to rest, the pressure falls to about 600 pounds. The cylinder is then removed from the rest press and the end cap is unscrewed and replaced by the die. The assembly is then placed in the die heater and mounted on the adapter cylinder. The die is kept warm in the die heater when not in use, and the equilibrium temperature while the dough is being extruded is kept at 105°F. The press is pumped up and the macaroni is extruded at a constant rate of 36 inches per minute. When the strand of macaroni reaches a length of 30 inches, it is cut and looped over round wooden sticks 4½ inches long and ¾ inch diameter. Four strands are obtained from the dough. These are allowed to case harden in the room for 5 minutes at a temperature of about 75°F and 35% relative humidity, after which they are placed in the drying cabinet maintained at 90°F and 95% R.H. The cabinet conditions remain at this level until one hour after the last sample of a batch is in, after which the humidity controller is set and the relative humidity falls at a uniform rate from 95% to 65% over a period of 48 hours. At the end of this time the heaters and humidifier are turned off and the cabinet is opened. The macaroni is left overnight with the fan running and thus reaches equilibrium with laboratory conditions.

The use of the rest press may be avoided by using an alternative method. The dough is placed in a beaker with a wax paper seal, and this is set for 30 minutes in a desiccator containing water. But this method eliminates the advantages of resting under pressure. The rest press may also be dispensed with by using the extrusion press for both functions, though this more than doubles the time required to process each sample.

Discussion

In working out a suitable method two ideas were kept mainly in mind. Firstly, although the method is a micro one and cannot begin to reproduce the conditions resulting from bulk handling in commercial processing, every attempt has been made to simulate commercial techniques at all stages in the processing. Secondly, the apparatus has been largely built in the laboratory workshop to keep expense at a minimum.

A thorough investigation has been made of all the essential stages of the micro processing to arrive at the method outlined, and some of the more important factors are discussed in the following paragraphs.

Cunningham and Anderson (1943) proposed working with a fixed absorption of 30% in processing discs, and this routine has also been adopted in the macro macaroni technique. It has been found, however, that a higher absorption is necessary for the micro method. With a single strand of macaroni, the effect of the fairly dry atmosphere (35% R.H.) of the laboratory is much more pronounced than when working with many strands. Moreover, when extruded through a heated die, the strand tends to crack readily when processed at 30% owing to the rapid surface drying. The optimum absorption for the micro method appears to be 31%, which produces a strand that is easy to handle and does not stretch if the room humidity is not above 45%.

It should be added, perhaps, that it might be better to work at a room humidity of 65% if this can be maintained, when a slightly lower absorption would be possible.

A fixed absorption is used for the micro test, primarily because material must be conserved. If adequate material is available it is undoubtedly better to work with more than one absorption level. This will show the tolerance of the samples to variations in absorption, and may indicate the level at which individual samples produce the best color.

The decision to rest the dough under pressure was influenced by two factors. The first of these is that this is actually done in the commercial process; the second is that, without this step, maximum efficiency cannot be obtained during the extrusion process. To obtain the best surface on the macaroni, the extrusion pressure should be fairly low. But to obtain a dough that requires comparatively little pressure for extrusion requires either an excessively long rest period, or a shorter rest period combined with pressure on the dough. The rest period was chosen first for convenience, as it fits in with the most efficient timing of the processing; and the optimum pressure for this time was then chosen with due regard to the limitations of the apparatus.

The effect of pressure during resting seems to be twofold: it appears to make the dough more translucent and it decreases its viscosity. The pressure required to extrude the macaroni at a constant rate is a direct function of the viscosity of the dough. Assuming that the die itself is in good condition, the surface of the macaroni depends on the extrusion pressure; the lower the extrusion pressure, the better the surface of the macaroni. Three factors are inversely related to the extrusion pressure: absorption, rest pressure, and die temperature. The values of these factors are all fairly high in the method chosen, and all contribute to a low extrusion pressure and thus improve the surface of the macaroni. The lower limit which can be placed on the extrusion pressure is that at which the dough ceases to weld properly after being forced through the three sections of the die.

It is desirable in testing a new method to compare its results directly with those obtained by commercial processing. It has not yet been possible to compare the macaroni made by the micro method with commercially made macaroni since the commercial method requires so much material that a special series of samples must be grown. Accordingly, it has been necessary to adopt as a standard of comparison the macro method, described by Binnington and Geddes (1936), which has been used in this laboratory. Nine varieties of durum wheats grown in Western Canada in 1945 were available for this study.

Macaroni was processed from these varieties by the two methods. Figure 7 shows a comparison of percentages of yellow and of white in the total reflected light for each of the samples. Agreement between the two methods is considered very good. The reflected red light is not shown as its value is low and is practically the same for all samples; the experimental error in its determination is also quite large, and the differences between samples are not significant.

The micro method is more reproducible than the macro method. This is readily understandable since the degree of control at all stages is much closer for the former than for the latter. In the micro method it is possible to control and vary all the conditions in the processing: absorption; mixing time and temperature; kneading time and type of kneading; rest time and pressure; extrusion time, pressure, and temperature; die temperature; and rate of drying. All these factors are directly under the control of the operator and all can be held within very close limits. The small size of the drying cabinet also aids in providing more uniform conditions for all samples. A series of 32 samples of macaroni, made from the same semolina and processed in

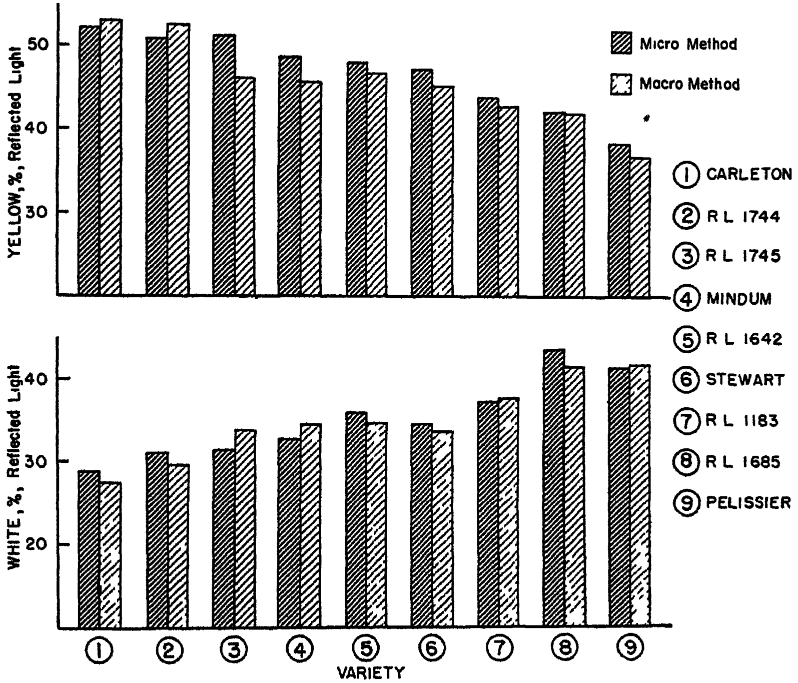


Fig. 7. Comparison of micro and macro methods showing reflected light scores for nine varieties of durum wheat.

one batch under identical conditions, had a standard error in pigment content of only ±.09 p.p.m. The surface of the micro macaroni is very similar to that of the commercial product, except that the former contains more air bubbles. This difference has been found with all small-scale tests so far reported and seems to be inherent in the necessity for shorter mixing times and subsequent more intimate and violent working of the dough.

The time required per sample in the micro method is less than half that required by the macro method and thus twice the number of samples can be processed in a given time. This facilitates the use of factorial type experiments and permits study of interactions between the various processing factors. A more thorough investigation, than has previously been possible in the laboratory, can now be made of the effects of the various processing conditions on macaroni quality.

Summary

Apparatus and method are described for making macaroni from 50-g samples of semolina. The dough is mixed at 31% absorption in a small motor-driven mixer with a U-shaped trough and horizontal spindle carrying four blades. It is then kneaded by passing it 10 times through a pair of rolls; the lower roll is free running and smooth, and the upper is motor-driven and corrugated with large blunt teeth. The dough is then placed in a small cylinder, with screw cap, and rested for 9 minutes under pressure in a Carver laboratory press. The cap is replaced by a single-holed die, maintained at 105°F by a heating plate, and the macaroni is extruded in the press at a rate of 36 inches per minute. Four 30-inch strands of macaroni are obtained and, after hardening in the room, they are dried for 48 hours in a small drying cabinet at 90°F with a controlled humidity gradient falling from 95% to 65%.

Data are presented which show excellent agreement between the new micro method and the macro method used in this laboratory. The reproducibility of the micro method is better than that of the macro method, and the quality of the macaroni from the former is equal to that of the latter method.

The apparatus was constructed in the laboratory workshop. It is compact and requires only about 7½ feet of bench space.

Literature Cited

Binnington, D. S., and Geddes, W. F.
1934 Experimental drying equipment for alimentary pastes. Can. J. Research 10: 221–233.
1936 Experimental durum milling and macaroni making technique. Cereal Chem. 13: 497–521.

Cunningham, R. L., and Anderson, J. A.
1943 Micro tests of alimentary pastes. I. Apparatus and method. Cereal Chem. 20: 171–185.
Fifield, C. C., Smith, G. S., and Hayes, J. F.
1937 Quality in durum wheats and a method for testing small samples. Cereal Chem. 14: 661–673.
Smith, G. S., Harris, R. H., Jesperson, E., and Sibbitt, L. D.
1946 The effect of pressure on macaroni discs: Size and number of air bubbles in relation to light transmission. Cereal Chem. 23: 471–483.

EFFECT OF GASSING RATE ON LOAF VOLUME [1]

H. Miller, J. Edgar, and A. G. O. Whiteside

Cereal Division, Central Experimental Farm, Ottawa, Canada

(Received for publication May 23, 1946)

The effect of gassing rate in the production of a well-risen loaf has been recognized since the earliest days of cereal science. Although Bailey (1916) put forward the gas production-retention theory, little was published on the subject by other workers for a number of years. Blish and Sandstedt (1927) concluded that fermentation tolerance was associated with the gas production factor. Jørgensen (1931) criticized the A.A.C.C. basic baking formula on the grounds of insufficient gassing power. In the period that followed, numerous workers investigated different aspects of gas production. In reporting their findings, most of the pertinent literature has been cited by such workers as Larmour and Bergsteinsson (1936); Eva, Geddes, and Frisell (1937); Sandstedt and Blish (1939); Eisenberg (1941); and Landis and Frey (1943).

Larmour and Brockington (1934) found that loaf volume is closely related to gas production in the proofing period; studies of gas rate curves and loaf volume showed that various estimations of baking strength would be obtained unless limiting conditions of fermentable sugar during the proofing period are eliminated, particularly with high protein experimentally milled flours.

The present paper deals with the gassing properties of experimentally milled flours of Canadian hard red spring wheat. The studies were undertaken to obtain a more intimate knowledge of the effects of supplementation with sucrose and diastatic malt.

Comparison of Inherent and Supplemented Gassing Rates

The gas production data were obtained with the automatic gas-recording apparatus described by the authors (1943). For the inherent gassing rates of the flour, the formula used was as follows:

---

[1] Contribution 138 of the Cereal Division, Dominion Experimental Farm, Ottawa, Canada.

	Grams	%
Flour	20.0	100.0
Yeast (bakers' wet compressed)	.6	3.0
Salt	.24	1.2
Water	11.0	55.0

The doughs were fermented for a 6-hour period in the gas-recording apparatus. The "inherent" gassing rate is the average of the three highest successive 10-minute periods of gas production. These peak rates represent the maximum gassing rates attained during the fermentation periods investigated.

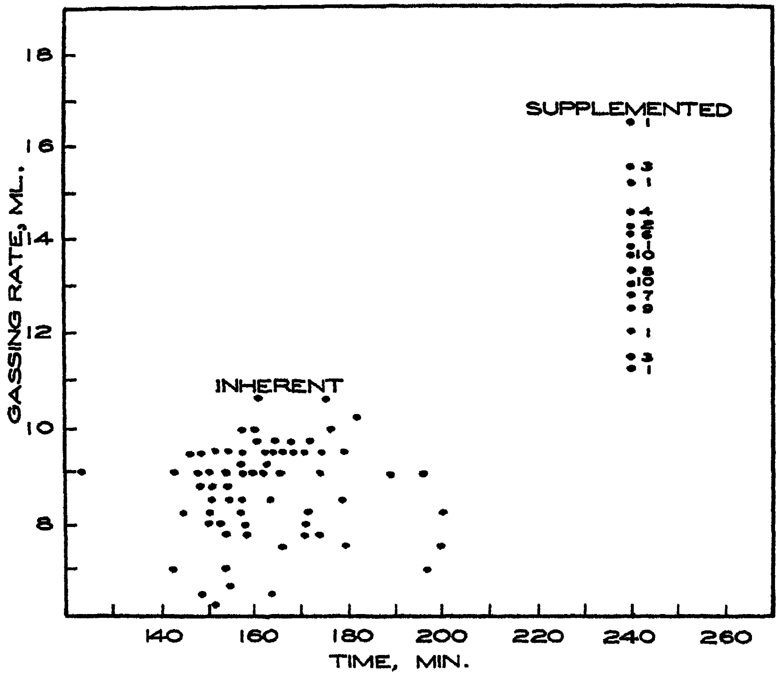


Fig. 1. Maximum "inherent" and "supplemented" gas production of 67 experimentally milled flour doughs. The "inherent" rates represent the mean peak gas production rates and the times at which they occur with doughs containing no added sugar or malt. The "supplemented" rates represent the mean gas production rates during the last 30 minutes of the pan proof period for corresponding doughs supplemented with 3.2% sucrose and 0.24% diastatic malt (250°L). The number of samples reaching the various rates is indicated.

The "supplemented" gassing data were obtained from the gas rates for 20 g flour doughs of the same formula as above plus a uniform supplement of 3.2% sugar and 0.24% diastatic malt (250°L). These "supplemented" doughs were fermented first in the fermentation cabinet according to the A.A.C.C. schedule (*Cereal Laboratory Methods*, 4th ed., 1941) and then placed in the gas-recording apparatus during the last 30 minutes of the pan proof period. The "supplemented" gassing figure is the mean of the three 10-minute periods of this 30-minute period.

"Inherent" and "supplemented" gassing values were obtained on 67 experimentally milled Canadian hard red spring wheat flours. These values are plotted in Figure 1. The data for "inherent" peak rates show wide variations not only in gassing rates but in the time at which the peak rates were produced. The "supplemented" gassing rates for the doughs at 210–240 minutes or the latter half of the final proof period show that the rates were higher for the "supplemented" doughs than for the "basic" doughs but that the amount of gas produced at oven time is not rendered uniform by the fixed addition of sucrose and malt. Individual increases in gassing rate varied from 1.5 to 8.5 ml. It is interesting to note that over two-thirds of the corrected values lie between 12 and 13.5 ml. However, it is demonstrated that flours vary unequally in their gas response to the fixed supplement and that the "supplemented" rates vary over as wide a range as do the "inherent" rates. Furthermore, it was found that the degree of increase in the "supplemented" rate is independent of the "inherent" rate.

Gas Production and Loaf Volume

The effect of gas production on loaf volume was investigated for a composite experimentally milled straight grade flour from Canadian hard red spring wheat. Four malt-~~sucrose~~ supplements and five fermentation time schedules were employed. Gassing rates and loaf volumes were determined for each combination in triplicate on three different days. A 700 g flour dough was made up each day using the following formula.

	Grams	%
Flour	700	100
Yeast (bakers' compressed)	21	3
Salt	7	1
$KBrO_3$	.007	0.001
$NH_4H_2PO_4$	.7	0.1
Water	448	64
Malt-sucrose solution	Varied	

The malt-sucrose solution was made up to contain 40 g sucrose and 3 g malt syrup (250°L) in 100 ml. The malt-sucrose solution additions employed were 1, 3, 5, and 8 ml per 100 g flour and corresponded to 0.4% sucrose +0.03% malt, 1.2% sucrose +0.09% malt, 2.0% sucrose +0.15% malt, and 3.2% sucrose +0.24% malt. The five fermentation times employed were 185, 205, 225, 245, and 265 minutes. The doughs were fermented for 105 minutes, punched and divided into five 100 g flour doughs for baking tests and ten 20 g flour doughs for gas production measurements. The doughs were panned at 130, 150, 170, 190, and 210 minutes, giving a total range in fermentation time of 80 minutes. The punching, panning, and baking schedules are shown below.

Total fermentation	Minutes				
	185	205	225	245	265
First punch	105	105	105	105	105
Second punch	—	125	145	165	185
Panning	130	150	170	190	210
Gassing tests begun	155	175	195	215	235
Into oven	185	205	225	245	265
Out of oven	210	230	250	270	290

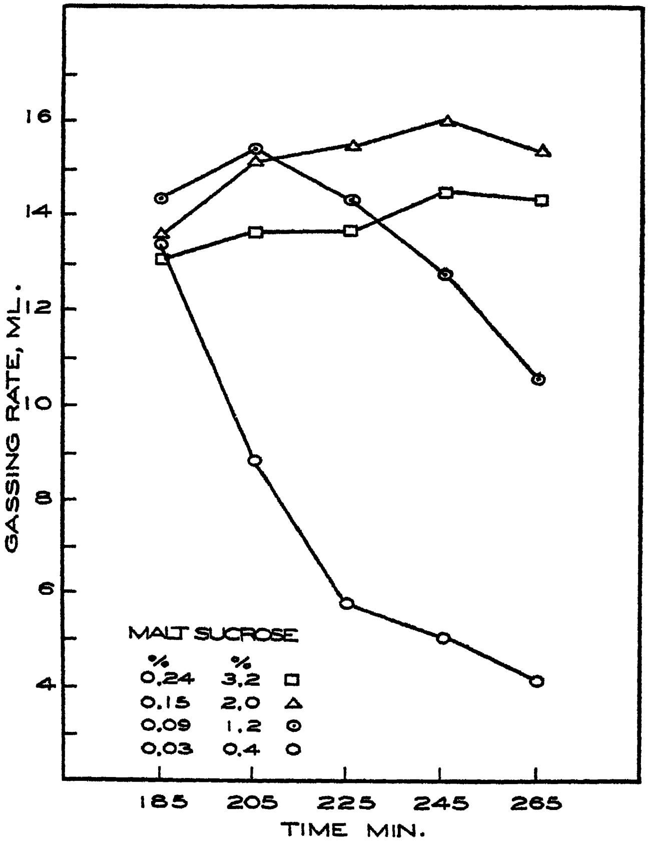


Fig. 2. Response in gassing rates to the addition of different amounts of sucrose and malt (250°L) at varying fermentation times in experimentally milled wheat flour doughs.

In Figure 2 the gassing rates for each malt-sucrose addition are plotted against fermentation time. At 185 minutes all four malt-sucrose supplements gave similar gassing rates ranging between 13 and 15 ml per 10-minute period for 20 g flour doughs. The 1 ml malt-sucrose solution addition showed a marked falling off in gas production as the fermentation time was extended. The 3 ml malt-sucrose solution doughs began to decrease at 245 minutes of fermentation and this was more pronounced at 265 minutes. The doughs containing 5 ml and 8 ml malt-sucrose solution remained high in gassing rate over all periods of fermentation time, but it is interesting to note that the former gave higher gassing rates than the latter at all fermentation times.

The relationship between gassing rate and loaf volume is shown in Figure 3. Low loaf volumes occurred when gas production rates were low, but they tended to level out as gas production reached a rate of 12 ml per 10-minute period (20 g flour dough).

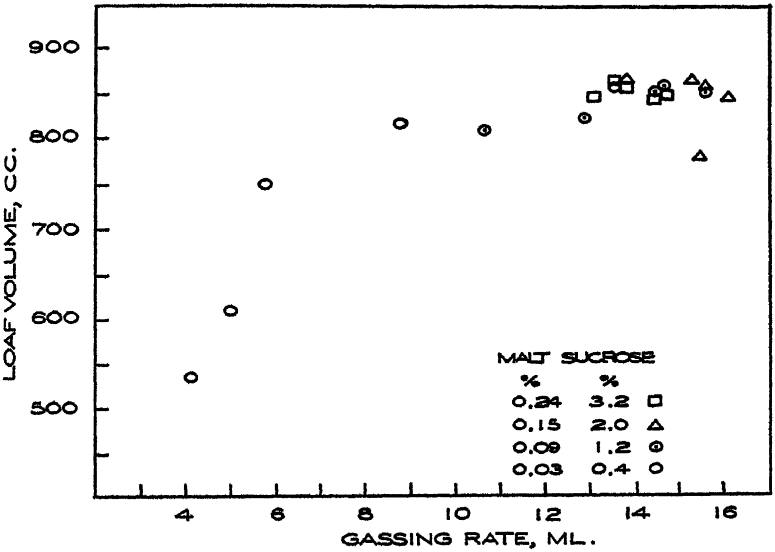


Fig. 3. The influence of gassing rate on loaf volume in experimentally milled wheat flour doughs containing different amounts of sucrose and malt (250°L).

Plotting loaf volume against fermentation time, it will be seen in Figure 4 that all malt-sucrose supplementations gave similar loaf volumes at 185 minutes and only the 1 ml malt-sucrose addition showed any marked falling off as fermentation time was extended. The 8 ml malt-sucrose solution maintained loaf volume rather better than the 3 ml and 5 ml supplements over the whole range of fermentation times. Since the loaf volume remained constant at all five fermentation periods the gassing rate obtained with the 8 ml addition would appear to be the most satisfactory for eliminating gas production as a factor influencing loaf volume. For this particular flour an equally satisfactory result was obtained by using a fermentation period of 185 minutes as with extended fermentation time. The most important factor was the amount of gas being produced in the latter part of the pan proof period.

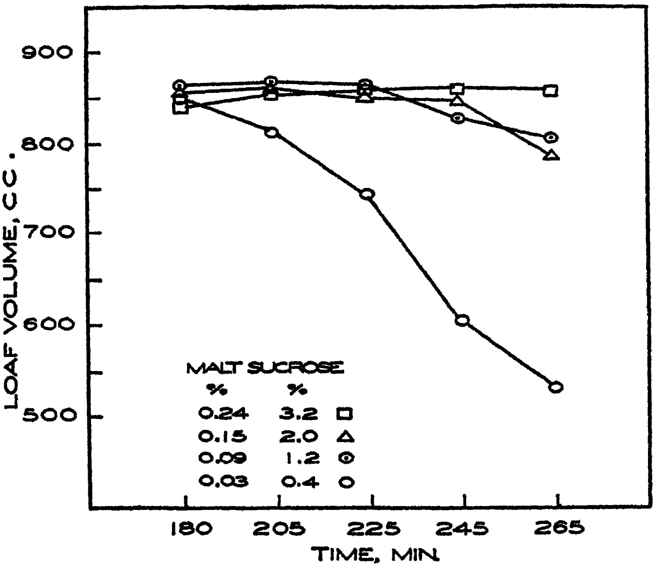


Fig. 4 The influence of fermentation time on loaf volume of experimentally milled wheat flour doughs containing different amounts of sucrose and malt (250°L)

Summary

"Inherent" gas production rates were determined on 67 experimentally milled Canadian hard red spring wheat flours. The gas production for these flours showed wide variations not only in peak rates but in the times at which these peaks occurred. The addition of sucrose and diastatic malt to the doughs increased the gassing rates as fermentation was extended to the pan proof period, but the degree of response was independent of the "inherent" rates.

The relationship of gas production and loaf volume was investigated on a composite experimentally milled flour using four different malt-sucrose supplements and five different fermentation times. Small additions of sucrose and diastatic malt gave high loaf volumes with short fermentation times, but larger additions gave uniform loaf volumes over a fermentation period extending from 185 minutes to 265 minutes. The maintenance of adequate gas production in the pan proof period resulted in high loaf volumes.

Literature Cited

Bailey, C. H.
1916 A method for the determination of the strength and baking qualities of wheat flour. Ind. Eng. Chem. 8: 53–57.

Blish, M. J., and Sandstedt, R. M.
1927 Factors affecting interpretation of experimental baking tests. Cereal Chem. 4: 291–299.

Eisenberg, S.
1941 Gas production in yeast fermentation and its applications. III. The baking test. Cereal Chem. 18: 267–299.

Eva, W. J., Geddes, W. F., and Frisell, B.
1937 A comparative study of various methods of measuring flour gassing power. Cereal Chem. 14: 458–480.

Jørgensen, H.
1931 On the separation of "gassing power" (diastatic activity) from "strength" in baking tests. Cereal Chem. 8: 361–374.

Landis, Q., and Frey, C. H.
1943 Direct determination of fermentation rates in dough. Cereal Chem. 20: 368–376.

Larmour, R. K., and Bergsteinsson, H. N.
1936 Studies on experimental baking tests. III. The effect of various salts on gas-production. Cereal Chem. 13: 410–420.

—— and Brockington, S. F.
1934 Studies on experimental baking tests. I. Effects of variation in baking formulas on gas production and loaf volume. Cereal Chem. 11: 451–470.

Miller, H., Edgar, J., and Whiteside, A. G. O.
1943 An automatic gas recording apparatus. Cereal Chem. 20: 355–361.

Sandstedt, R. M., and Blish, M. J.
1939 The question of sugar levels in laboratory baking. Cereal Chem. 16: 36–42.

ANALYSIS OF DATA FOR A.A.C.C. CHECK SAMPLE SERVICE. II. PROTEIN AND THIAMINE RESULTS, 1944-45 [1]

W. O. S. Meredith

Grain Research Laboratory, Board of Grain Commissioners
for Canada, Winnipeg, Manitoba

(Received for publication June 27, 1946)

In summarizing the data obtained in the National Check Sample Service during 1944–45, similar methods were used to those reported by Meredith (1945) and the reader is referred to that paper for details.

Protein

A list of the samples submitted for analysis, together with the mean protein contents, follows:

Sample	Mean protein, %
1. Soft wheat flour	8.18
2. Fine ground whole wheat flour	14.42
3. Low protein family flour	10.67
4. Hard wheat enriched flour	10.70
5. Low grade Red Dog flour	13.06
6. Soft wheat cake flour, enriched	7.02
Mean	10.69

---

[1] Published as paper No. 87 of the Grain Research Laboratory.

Differences between Laboratory Means over Six Samples. In order that collaborators may know how the means for their own laboratories compare with the general mean over all laboratories, which is 10.69%, differences between means for each laboratory and the general mean have been listed in Table I. The laboratories have been listed in order of decreasing negative differences by columns to the lower part of column 4, where the difference is zero, and thereafter in order of increasing positive difference.

TABLE I

Differences between Laboratory Mean for Protein Contents of Six Samples and General Mean for All Laboratories

(Laboratories are identified by the first number in each column)

Range of difference from general mean													
−.25		−.16 to −.07		−.06 to −.04		−.03 to +.02		+.04 to +.06		+.07 to +.15		+.25 to +.37	
Lab. No.	%	*Lab. No*	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%
129	−.25	79	−.16	20	−.06	83	−.03	27	+.04	87	+.07	18	+.25
		110	−.15	63	−.06	100	−.03	12	+.04	51	+.07	73	+.26
		13	−.13	94	−.05	42	−.03	70	+.05	68	+.09	97	+.37
		85	−.13	127	−.05	74	−.03	82	+.06	72	+.09		
		104	−.13	17	−.05	33	−.03	16	+.06	28	+.10		
		123	−.09	19	−.05	39	−.02			64	+.11		
		125	−.09	32	−.04	105	−.02			89	+.11		
		35	−.08	75	−.04	107	−.02			56	+.12		
		49	−.08	6	−.04	22	−.02			78	+.14		
		86	−.08	76	−.04	43	−.02			93	+.15		
		24	−.08	120	−.04	92	−.02						
		103	−.07			3	−.01						
		1	−.07			47	−.01						
		13	−.07			108	−.01						
		29	−.07			124	−.01						
		53	−.07			58	0.00						
						5	0.00						
						54	0.00						
						118	0.00						
						128	0.00						
						45	0.00						
						98	0.00						
						48	+.01						
						81	+.02						

The 24 laboratories in the center column are those having means within ±0.03 of the general mean, which is beyond criticism. In the adjacent columns (3 and 5) are the additional 16 laboratories with means within ±0.06% of the general mean, which is good.

The remaining columns require further consideration. The first and last columns contain four laboratories with means differing by ±0.16% or more from the general mean. These are classed as unsatisfactory because statistical analyses show that a difference of ±0.16% from the general mean is significant at the 5% level. Subsequent examination of the data showed that the standard error of a single sample (0.19%) was abnormally high and that the "normal" necessary difference was 0.07%. On this latter basis, the 26 laboratories in the second and second-last columns, which report differences from ±0.07 to ±0.16%, must be classified as questionable.

Accordingly, of the 70 laboratories, 40 reported mean values over the six samples that are satisfactory, 26 reported results that are of questionable accuracy, and the remaining four reported results that are classified as definitely erroneous. The corresponding figures for the 1943–44 Check Test Series were 38 satisfactory, 18 questionable, and 12 definitely erroneous, out of 68 laboratories.

Differences between Laboratory Means for Single Samples. An examination of the laboratory means for each individual sample showed standard errors for the six samples of 0.13, 0.27, 0.28, 0.10, 0.20, and 0.14%. Detailed examination showed that the distributions of values for all samples except No. 4 were abnormal, and, after eliminating the 11 values causing the abnormality, the adjusted standard deviations for the samples dropped to 0.09, 0.15, 0.11, 0.10, 0.11, and 0.12%. The pooled standard error of a single determination for the six samples then becomes 0.11%. The corresponding figure obtained last year for five samples was 0.10%.

TABLE II

STANDARD ERROR OF EACH LABORATORY OF DIFFERENCE BETWEEN LABORATORY MEAN AND GENERAL MEAN

(Laboratories are identified by first number in each column)

Range of standard errors of differences							
0.82 to 0.13		0.12 to 0.10		0.09 to 0.07		0.06 to 0.01	
Lab. No.	%	*Lab. No.*	%	*Lab. No.*	%	*Lab. No.*	%
97	.82	51	.12	123	.09	125	.06
110	.62	39	.12	86	.09	3	.06
78	.53	76	.12	107	.09	127	.06
73	.52	124	.12	17	.09	22	.06
16	.32	42	.12	43	.09	98	.06
129	.27	48	.12	72	.08	104	.06
35	.27	89	.12	128	.08	19	.05
113	.23	87	.11	94	.08	103	.05
74	.21	33	.11	108	.08	81	.05
28	.18	70	.11	32	.08	58	.05
63	.17	64	.11	6	.07	53	.05
18	.15	92	.11	13	.07	100	.05
24	.15	79	.10	12	.07	83	.04
85	.15	120	.10	27	.07	29	.04
75	.14	68	.10	45	.07	105	.04
54	.13	56	.10			1	.04
93	.13	5	.10			47	.03
		82	.10			49	.01
		20	.10				
		118	.10				

Variability within Laboratories. Differences between laboratory means over six samples do not give an account of the intralaboratory errors. Thus two laboratories might have mean differences of zero, yet one might have had +0.1 for three samples and −0.1 for the other three, while the other had differences of 0.0 for all six samples. The consistency of the laboratories can be compared by listing the standard errors of the mean difference from the general mean for each laboratory, and these are given in decreasing order in Table II. The mean standard error within laboratories was 0.19% and the range was from 0.01 to 0.82%. Statistical analyses showed that there were significant differences between the standard errors and that the 17 values listed in the first column of Table II can be considered abnormal. When these are removed, the mean standard error of the remainder is 0.09%, which is identical with the value obtained last year.

Discussion. Comparison of the results obtained this year with those obtained last year suggests that there has been some slight improvement in the data for protein content.

Thiamine

Only 19 laboratories reported thiamine results for all six samples. The differences between the general mean and the laboratory mean for each laboratory are given in Table III. Last year it was found that the mean values for the laboratories ranged from 2.39 to 3.74 mg/lb,

TABLE III

COMPARISONS AMONG LABORATORIES FOR MEAN DIFFERENCE FROM GENERAL MEAN AND STANDARD ERROR OF DIFFERENCE FOR THIAMINE CONTENT IN MG/LB OF SIX SAMPLES OF FLOUR

Lab.	Mean difference from general mean	Standard error of difference	Lab.	Mean difference from general mean	Standard error of difference
	mg/lb	*mg/lb*		*mg/lb*	*mg/lb*
24	−0.25[1]	0.10	100	+0.04	0.08
16	−0.17[1]	0.15	117	+0.06	0.10
94	−0.16[1]	0.13	5	+0.08	0.20
6	−0.11	0.11	123	+0.08	0.39[2]
89	−0.11	0.22	108	+0.09	0.10
63	−0.09	0.15	13	+0.12[1]	0.10
92	−0.06	0.08	51	+0.12[1]	0.09
22	−0.06	0.14	20	+0.24[1]	0.18
125	0.00	0.13	78	+0.24[1]	0.13
113	+0.01	0.21			

General mean over all laboratories and samples.............. 2.19 mg/lb
Original mean standard error of single sample............... 0.17 mg/lb
Normal mean standard error of single sample after reduction
from heterogeneous series............................ 0.14 mg/lb

---

[1] Significantly different from general mean.
[2] Significantly different from normal standard error.

a difference of 1.35 mg/lb. This year the laboratory means ranged from 1.94 to 2.43, a difference of 0.49 mg/lb, which represents a substantial improvement.

The standard error of a single sample was 0.52 mg/lb last year and 0.17 mg/lb this year. With the removal of the results of the laboratory which showed the greatest variability, the standard error was reduced to 0.14 mg/lb as compared to the corresponding figure of 0.30 mg/lb last year. On the basis of a standard error of 0.14 mg/lb, the necessary difference from the mean is 0.12 mg/lb, and the seven laboratories marked with footnote 1 in Table III may be considered to have given questionable mean values. The variability within laboratories was examined and the standard error of the laboratory's mean difference from the general mean was included in Table III. Only one laboratory (marked with an asterisk) was shown to give an abnormal standard error for the difference. This again compares favorably with last year when seven laboratories gave abnormal standard errors.

TABLE IV
Mean, Standard Deviation, and Coefficient of Variability for Each Sample for Thiamine mg/lb

Sample	Mean thiamine	Degrees of freedom	Standard deviation	Coefficient of variability
	mg/lb		*mg/lb*	%
1	1.67	18	0.22	13.2
2	2.05	18	0.21	10.2
3	2.07	18	0.15	7.2
4	2.03	18	0.15	7.4
5	3.07	18	0.24	7.8
6	2.22	18	0.22	9.9
Mean	2.19		0.20	

The standard deviations within samples are shown in Table IV; these were found to be normal. In general, the coefficients of variability for the six samples show considerably greater uniformity than was attained with the six samples studied in 1943–44. This also indicates improvement in the determination as a whole.

Discussion. The improvement achieved during the past year in the thiamine determination should be extremely encouraging to all those who have worked on this determination. Its precision is not yet of the order of that achieved in such standard determinations as that of protein, but there is every reason to believe that a further improvement will be shown next year, and in time the precision of the thiamine determination may well rank with that of other standard methods.

Summary

Seventy laboratories analyzed all six of the 1944–45 A.A.C.C. Check Samples for protein content, and differences between laboratory means were demonstrated. The original standard error of a single sample was 0.19% protein, and 94% of the laboratories agreed within ±0.16% of the general mean value of 10.69% protein for the six samples. The standard error was found to be abnormal and the normal standard error was 0.09% protein; 57% of the laboratories agreed within ±0.07% of the mean protein value. The variability within laboratories was also examined and 17 laboratories showed abnormally high variability.

The data for the thiamine determination, which represent results from 19 laboratories, also showed differences between laboratory means. The original standard error of a single sample was 0.17 mg/lb thiamine and 72% of the laboratories agreed within 0.14 mg/lb of the general mean value of 2.19 mg/lb thiamine for the six samples. This standard error was abnormal and was reduced to 0.14 mg/lb by removal of the result for one laboratory that showed high variability; 63% of the laboratories agreed within 0.12 mg/lb of the mean thiamine value.

The protein results show a slight improvement over those obtained in the previous year, and the thiamine results show a substantial improvement over those for the previous year both in agreement between laboratories and in intralaboratory variability.

Acknowledgment

The interest of Dr. J. Ansel Anderson, Chief Chemist of this laboratory, in the study and his suggestions during preparation of the manuscript are gratefully acknowledged.

Literature Cited

Meredith, W. O. S.
1945 Analysis of data for A. A. C. C. check sample service. I. Protein and thiamine results, 1943–44. Cereal Chem. 22: 437–448.

MODIFICATION OF THE GAS PRODUCTION AND GAS RETENTION PROPERTIES OF DOUGH BY SOME SURFACE-ACTIVE, REDUCING, AND OXIDIZING AGENTS[1]

EMERY C. SWANSON[2] and C. O. SWANSON

Kansas State College, Manhattan, Kansas

(Received for publication May 7, 1946)

The modification of wheat flour dough by chemical means is of importance, especially since wheat varieties are raised which are superior in agronomic properties but whose flours make doughs of inferior handling properties. Swanson and Andrews (1942, 1944, 1945) studied the influence of surface-active and reducing agents on the behavior of dough in mixing. They found that the surface-active agents increased the optimum mixing time and decreased the effects of overmixing, whereas reducing agents had the opposite effect. Thus, Chiefkan flour was modified by these wetting agents to produce mixogram patterns similar to those obtained from Turkey or Tenmarq flours; and, likewise, reducing agents produced mixogram patterns from Tenmarq flour similar to those from Chiefkan flour.

---

[1] Contribution No. 131, Department of Milling Industry.
[2] Present address: International Milling Company, Minneapolis 1, Minnesota.

A review of the literature regarding the effect of oxidizing and reducing agents on wheat flour has been given in a paper by Shen and Geddes (1942). A supplemental review which brings the literature citations up through 1945 was made by Howe (1946).

In addition to their effects on mixing characteristics, the influence of chemicals on gas production and gas retention is of importance in breadmaking. This paper reports experiments on the influence of surface-active, reducing, and oxidizing agents on gas production and gas retention.

Materials and Methods

The recording apparatus described by Working and Swanson (1946), which provides continuous gas production and gas retention data, was used.

A commercial bakers' hard red winter wheat flour (11.9% protein, 0.44% ash, and 11.4% moisture) and a "lean" formula (flour, water, 2.5% yeast) was used throughout. The only variations were in the kind and amount of chemical agent added.

The surface-active agents used were Aerosol OT,[3] Santomerse S,[4] and Tergitol penetrant 08.[5] The reducing agents used were cysteine hydrochloride, glutathione, and sodium sulfide, and the oxidizing agents, potassium iodate, potassium bromate, and hydrogen peroxide. Ten concentrations of each agent were employed, in addition to the controls. The range of concentrations varied in equal intervals from 60 to 600 mg per 100 g of wheat flour for the surface-active agents, from 6 to 60 mg for the reducing agents, and from 3 to 30 mg for the oxidizing agents. These ranges were sufficiently wide to insure uncovering any effect that might result from the addition of these agents, and are similar to the concentrations used by Swanson and Andrews (1942, 1944, 1945) in their mixogram studies.

A uniform mixing time of two minutes was used for each dough. This period was established as optimum for the flour without the addition of any of the chemical agents investigated. A uniform period would eliminate the influence of mixing time and, consequently, not interfere with the dough development factor except as the mixing requirements were modified by the agent used. A 10-g aliquot was used to determine gas production and another one for the determination of gas retention. The 90 combinations, 9 chemicals and 10 concentrations of each, were made in triplicate, along with 27 controls. The sequence of the combinations and control samples, during the course of the experiment, was by random selection.

---

[3] American Cyanamid & Chemical Corporation, New York, N. Y.
[4] Monsanto Chemical Company, St. Louis, Missouri.
[5] Union Carbide and Carbon Corporation, New York, N. Y.

The gas production data have been reported as "initial rate of production" (ml/hour/170 g dough[6]) and "duration of the initial rate" (hours) and the gas retention data as the "maximum amount of gas retained" (ml/170 g dough) and the "time to reach the maximum" (hours). The initial rate of gas production is the average rate during which an adequate supply of fermentable sugar is present and is maintained until the supply becomes so small as to be the limiting factor in fermentation. This stage is shown by an abrupt decrease in the slope of the production curve. The *time* elapsing from the start of fermentation to the change in the slope is the "duration of the initial rate." Maximum gas retention occurs when the rate of production is equalled by the rate of escape from the dough. Prior to maximum retention the rate of production exceeds the rate of loss and the *amount* retained is increasing. After the point of maximum retention has been reached the rate of production has been surpassed by the rate of escape and the amount of gas retained is decreasing. Maximum retention is represented by the highest point on the retention curve (Working and Swanson, 1946). The time elapsing from the start of fermentation to this point is the "time to maximum retention."

Results

For convenience in presenting and discussing the four phenomena studied, namely, initial rate of fermentation, duration of initial rate, maximum gas retention, and time to maximum retention, they will usually be referred to as "rate," "duration," "retention," and "time," respectively. The mean data are shown graphically in Figures 1, 2, and 3. The significant correlations for the effects of the amount of the chemical agents on these four factors have been listed in Table I. Of 36 possible correlations, 13 were nonsignificant, 5 were significant, and 18 were highly significant.

All but five of the 23 significant relationships indicated that the effects of the chemical agents were directly proportional to their con-

---

[6] 170 g being the average weight of an unfermented pup-size dough.

TABLE I

SIGNIFICANT CORRELATION COEFFICIENTS FOR THE EFFECTS OF CHEMICAL AGENTS ON (a) INITIAL RATE OF PRODUCTION, (b) DURATION OF INITIAL RATE, (c) MAXIMUM AMOUNT OF GAS RETAINED, (d) TIME TO REACH THE MAXIMUM

Chemical agents	Correlation coefficients			
	Production		Retention	
	Initial rate	Duration of initial rate	Maximum amount	Time to maximum
Aerosol OT	−0.736	—	−0.959	−0.905[1]
Santomerse S	—	−0.862	−0.892	—
Tergitol pen. 08	−0.892	+0.612	—	+0.822
Cysteine hydrochloride	—	—	−0.950[1]	−0.830[1]
Glutathione	−0.843	+0.640[1]	−0.838	−0.853
Sodium sulfide	—	−0.859	−0.791	−0.835
Potassium iodate	—	—	—	—
Potassium bromate	−0.692	+0.707	+0.860[1]	+0.929
Hydrogen peroxide	+0.624	—	−0.908	—

NOTE: 0.602 = r at 5% level
0.735 = r 1% level.

[1] Curvilinear correlation requiring:
0.632 at 5% level
0.765 at 1% level.

centration. Of the five, cysteine produced a maximum effect on time and bromate a maximum effect on retention; Aerosol caused an accelerated decreasing effect on time and cysteine on retention beyond a certain concentration; glutathione caused an accelerated increasing effect on duration.

Figure 1 shows the effects of the surface-active agents, Aerosol, Santomerse, and Tergitol, on gas production (rate and duration), retention, and retention time. Aerosol markedly lowered the rate and, from the graph, seemed to have a decreasing influence on duration which was, however, not substantiated by regression analysis. Retention was lowered but time was not decreased until the concentration exceeded 0.3 g Aerosol per 100 g flour. Santomerse had no influence on production rate or retention time, but markedly decreased duration of the rate and maximum retention. Tergitol decreased the rate, increased duration and time, but was without influence on retention.

The effects of the reducing agents are shown in Figure 2. Cysteine had no effect on gas production but it decreased gas retention. Cysteine exhibited an optimum concentration in affecting retention time, although the increase on retention time at the lower concentrations was not as marked as the decrease at the higher concentrations.

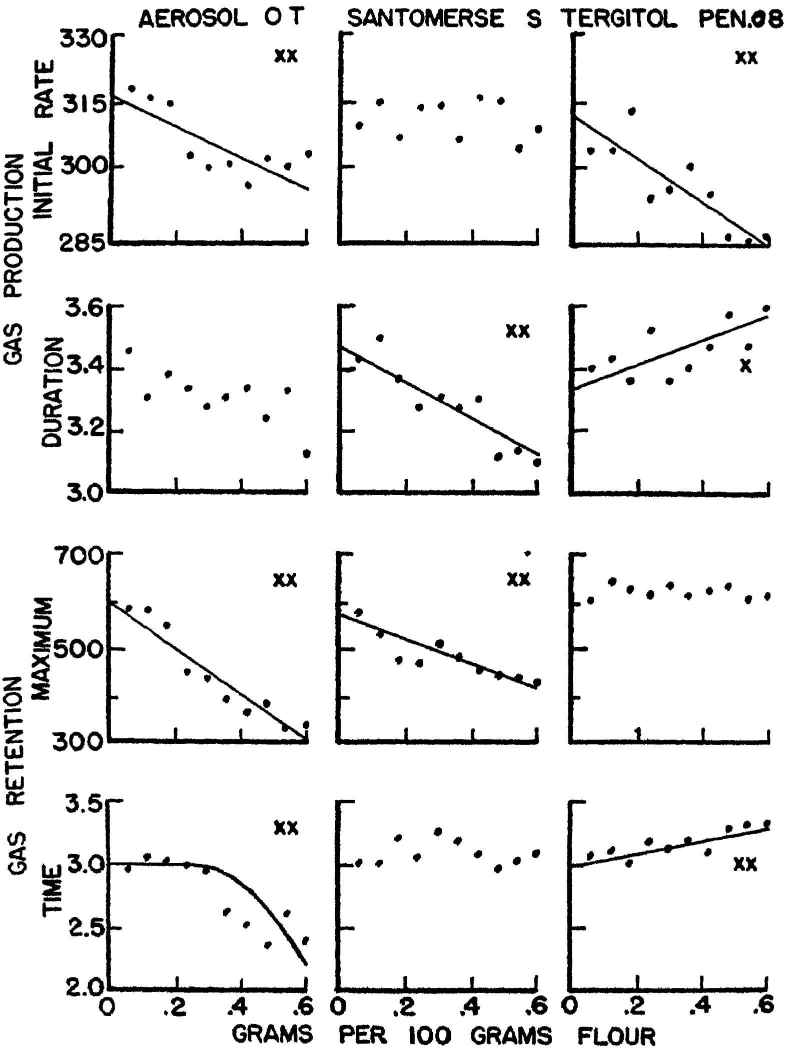


Fig. 1. Effect of surface-active agents on gas production and gas retention. Initial rate is given in milliliters per hour per 170 g dough, maximum retention in milliliters per 170 g dough, and duration of rate and time to maximum retention in hours. Regression lines are shown for all significant relationships.

Glutathione decreased the rate, retention, and time; but increased the duration of gas production rate when the concentration was sufficiently great. Sodium sulfide had no influence on production rate, but decreased the duration. This reagent caused a slight decrease in maximum retention and markedly decreased retention time.

The effects of the oxidizing agents are shown in Figure 3. Potassium iodate was entirely without influence on gas production and gas retention. Potassium bromate, according to the regression equation, had a depressing action on production rate but the positions of the points indicate some doubt. The duration was increased slightly by bromate and an optimum maximum retention was attained with about 15 mg per 100 g flour; the time to maximum retention was also increased as the dosage of bromate was increased. Hydrogen peroxide apparently increased the rate of production, but had no influence on

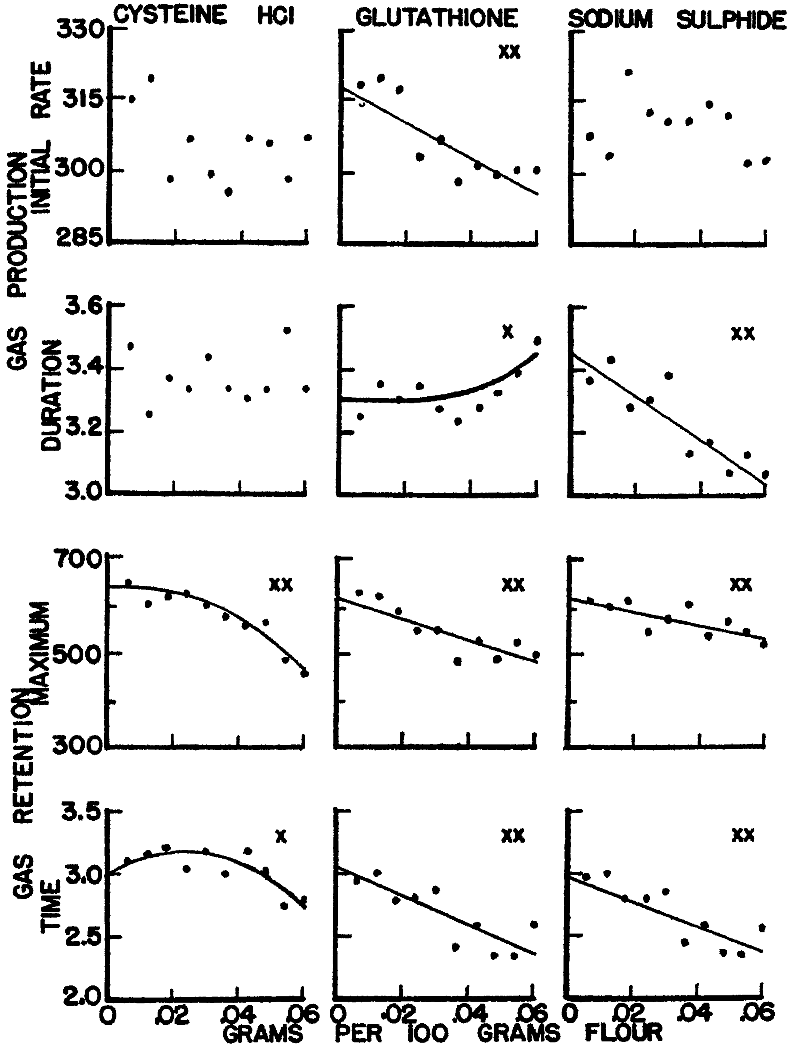


Fig. 2. Effect of reducing agents on gas production and gas retention. Initial rate is given in milliliters per hour per 170 g dough, maximum retention in milliliters per 170 g dough, and duration of rate and time to maximum retention in hours. Regression lines are shown for all significant relationships.

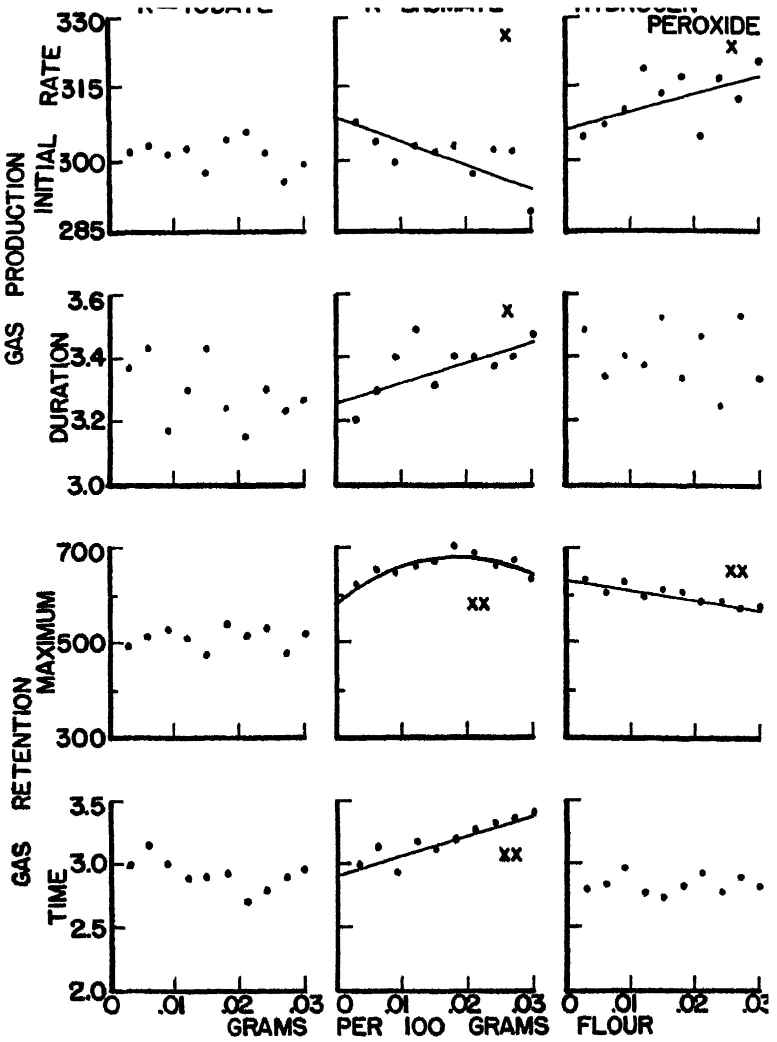


Fig. 3. Effect of oxidizing agents on gas production and gas retention. Initial rate is given in milliliters per hour per 170 g dough, maximum retention in milliliters per 170 g dough, and duration of rate and time to maximum retention in hours. Regression lines are shown for all significant relationships.

duration. It decreased retention but had no effect on the time to maximum retention.

Discussion

Although the results reported in this paper are preliminary, some general observations on the action of the agents used on gas production and gas retention may be made.

Gas Production. Two factors are predominant in gas production, namely, enzymic action and starch susceptibility. The enzymic activities are divisible into two groups, those which hydrolyze starch to sugar and those which ferment sugar. A decrease in the initial rate of gas production would indicate an inhibiting effect on yeast growth and/or the fermentation enzymes inasmuch as adequate sugar was apparently present. An increase in rate, conversely, would indicate an accelerating effect. Of the surface-active agents, Aerosol and Tergitol had an inhibiting effect on rate but Santomerse was without effect. Of the reducing agents, glutathione was the only one which inhibited the rate of production of gas; cysteine and sodium sulfide were inactive. Of the oxidizing agents, the bromate was depressing and the peroxide accelerating, but the iodate was inactive.

A change in the duration of the initial rate of fermentation could result from either, or both, of two actions: an inhibiting action on the starch hydrolyzing enzyme system and/or a change in the starch susceptibility. Aerosol did not have any significant effect on duration, but Santomerse decreased it and Tergitol caused an increase. Of the reducing agents, cysteine had no effect on duration, sodium sulfide shortened it markedly, and beyond the medium concentration used, glutathione increased the duration. Of the oxidizing agents, bromate lengthened the duration, but iodate and peroxide were without effect.

Gas Retention. Gas retention differs from gas production in that an adequate dough structure is requisite for gas retention, but not for gas production. Amount of mixing has a marked influence on such a structure (Swanson and Swanson, 1946), but all doughs were given the same mixing treatment in this study in an attempt to determine the effects of chemical agents without the interference of the mechanical dough-development factor.

Chemicals that influence gas retention are effective, primarily, through their capacity to alter the physical structure of the dough. In addition, the rate of gas production influences gas retention; an increase in the rate of production increases the amount of gas retained owing to the greater rate of escape necessary before the loss balances production. Hence, a chemical which increases or decreases the rate of production would increase or decrease the maximum gas retention.

A part of the decrease in gas retention from Aerosol was due to the decrease in rate of production. However, the decrease in rate cannot account for all the loss in retention. If all the loss in retention were attributable to decreased rate, fermentation would have to continue for about 15 hours, with the maximum retention coming at that time (20 ml/hour rate decrease; 300 ml retention decrease), but no fermentation period was continued for more than 8 hours, and the time to maximum retention in no case exceeded 3½ hours. This indicates that Aerosol had an appreciable detrimental effect on the dough structure, with respect to the gas-retaining capacity. A similar effect is attributable to Santomerse; the decrease in gas retention was not quite as great although Santomerse was without influence on the rate of production. Therefore, the detrimental effect of Santomerse apparently was about the same as that of Aerosol. The gas-retaining ability of dough was seemingly not affected by Tergitol. However, since a marked decrease in the production rate occurred without any concurrent decrease in gas retention, it is logical to assume that were the production rate maintained constant, Tergitol would show an increase in maximum gas retention.

Most of the detrimental effect of glutathione on gas retention can be accounted for by its depressing action upon the rate of gas production. Since cysteine and sodium sulfide had no influence on gas production rate, their effect on gas retention must be interpreted as due largely to some harmful alteration in dough structure.

Of the oxidizing agents, bromate appears to exhibit an optimum concentration in relation to maximum gas retention. Bromate improved gas retention to a marked extent and when compensation for the seeming reduction in production rate is made, the bromate curve indicating increased retention becomes even more significant. The detrimental effect of hydrogen peroxide on maximum retention was actually more marked than it appears from the graph, since hydrogen peroxide apparently increased the rate of gas production.

Interrelations of Gas Production and Gas Retention. The time to maximum retention is dependent upon both the rate of gas production and the dough structure. Tergitol and potassium bromate had no effect on dough structure, as measured by the gas-retaining capacity of the dough, yet they caused an increase in the time to maximum retention. Aerosol seemed to affect the time factor through both the initial rate of gas production and the retention effects; however, no decrease in time to maximum retention occurred unless the concentration of Aerosol exceeded the medium amount used. On this basis it seems probable that Aerosol would increase retention time up to a certain concentration, if production rate and maximum retention were maintained constant. Santomerse appeared to have no influence on the time to maximum retention in spite of a definite harmful influence upon dough structure, as indicated by a decrease in maximum retention.

With cysteine, an optimum concentration for the time to maximum retention was noted. The decreasing effect of cysteine on time as the concentration was increased beyond the optimum was abetted by its harmful effect on dough structure, as measured by maximum retention. The decrease in gas retention due to glutathione was apparently caused by both a decrease in the rate of gas production and by an unfavorable effect on dough structure. Sodium sulfide reduced gas retention principally by its harmful action on dough structure.

Of the oxidizing agents, iodate and peroxide had no effect upon retention time, but bromate markedly increased the time, in spite of causing a decrease in rate of gas production. The evidence indicates that bromate has an appreciable influence in the improvement of the dough structure for gas-retaining purposes.

Summary

A commercial bakers' hard red winter wheat flour and a flour-water-yeast formula were used to study the effects of surface-active, reducing, and oxidizing agents on gas production and gas retention. The surface-active agents were Aerosol OT, Santomerse S, and Tergitol penetrant 08; the reducing agents were cysteine, glutathione, and sodium sulfide; and the oxidizing agents were potassium iodate, potassium bromate, and hydrogen peroxide.

These preliminary studies have shown variable effects of the agents used on both gas production and retention. The initial rate of gas production was decreased by Aerosol, Tergitol, glutathione, and apparently bromate; and increased by peroxide. The duration of the initial rate was decreased by Santomerse and sodium sulfide, and increased by Tergitol, bromate, and glutathione (beyond a certain concentration). The maximum gas-retaining capacity of dough was decreased by Aerosol, Santomerse, cysteine, glutathione, sodium sulfide, and hydrogen peroxide, but was increased by bromate (up to a certain concentration). The time elapsing from the start of fermentation until maximum gas retention was reached was decreased by Aerosol (beyond medium concentration), glutathione, and sodium sulfide; and increased by Tergitol and bromate. Cysteine increased the time factor up to medium concentration, but beyond that it caused a decrease.

Literature Cited

Howe, M.
1946 Further studies on the mechanism of the action of oxidation and reduction on flour. Cereal Chem. 23: 84–88.

Shen, T., and Geddes, W. F.
1942 Protease activity and reducing matter content of wheat flour doughs in relation to baking behavior. Cereal Chem. 19: 609–631.

Swanson, C. O., and Andrews, A. C.
1942 Factors which influence the physical properties of dough. IV. The effects of surface active agents on the characteristics of the curves made by the recording dough mixer. Cereal Chem. 19: 102–120.

1944 Factors which influence the physical properties of dough. VI. Effect of cysteine and some other substances on mixogram patterns. Cereal Chem. 21: 140–149.
1945 Effects of papain, yeast water, cysteine, and glutathione on gluten dispersion or on disintegration as indicated by gluten recovery and by mixogram patterns. Cereal Chem. 22: 134–149.
Swanson, E. C., and Swanson, C. O.
1946 Gas production and gas retention of dough as affected by type of flour, baking formula, and amount of mixing. Cereal Chem. 23: 432–440.
Working, E. B., and Swanson, E. C.
1946 An automatic device for the measurement of gas production and gas retention in doughs. Cereal Chem. 23: 210–216.

DETERMINATION OF UREA AND AMMONIACAL NITROGEN IN FEEDS

Margaret Schmoll and Frederick D. Schmalz

Russell-Miller Milling Company, Control Laboratory, Minneapolis, Minnesota

(Received for publication March 29, 1946)

Because of the shortage of all types of protein concentrates during the past few years, urea has been used to a greater extent than ever before in dairy and sheep commercial mixed feeds. Upon the recommendation of the Association of American Feed Control Officials, the use of urea and certain ammonium salts has been limited to not more than one-third of the total crude protein nitrogen in feeds for ruminants.

Under the direction of the Associate Referee, W. B. Griem, the Association of Official Agricultural Chemists has published several reports on a method for the determination of urea and nitrogen salts in feeds (Griem, 1941, 1941a, 1942, 1942a, 1944). A recommendation (Griem, 1944) was made that the method as revised (1942) be made official (final action).

In our hands some difficulty was encountered in using this method in routine analyses of urea-containing feeds, particularly those concentrates that had relatively high amounts of urea. Excessive foaming was obtained in the distillation and an unsatisfactory fleeting end point was experienced in the titration. Therefore, certain minor modifications in the method were introduced which make the procedure more reliable. These modifications are essentially the use of sodium hydroxide, a larger amount of calcium chloride, and the introduction of material to prevent foaming and bumping. Glass beads and a small quantity of finely ground mill feed (shorts) served the purpose better than anything else that was tried. The mill feed used for this purpose should, of course, contain no added urea or ammonium salts.

Determination

Reagents:

Calcium chloride: 25% solution
Sodium hydroxide: 10% solution
Urease tablets: 25 mg (a product of Hynson, Wescott, and Dunning, Inc.)
Buffer solution: 41.0 g of sodium acetate and 30.0 g glacial acetic acid per liter
Standard acid: Normality as used in the Kjeldahl nitrogen determination
Standard alkali: Normality same as standard acid
Indicator: Sodium alizarin sulfonate or methyl red
Paraffin
Glass beads
Finely ground mill feed (shorts).

Procedure:

Weigh a 10.0 g sample of the urea-containing feed, place in a 500 ml volumetric flask, and make up to volume. Allow the suspension to settle after shaking and pipette 20 ml of the supernatant liquid into an 800 ml Kjeldahl flask. Add buffer in amounts corresponding to the following levels of urea: 1 to 5% urea—1.2 ml buffer solution; 6 to 10% urea—2.4 ml buffer solution; 11 to 15% urea—3.6 ml buffer solution. Grind two urease tablets in a glass mortar and wash into the flask with not more than 100 ml of water. Agitate gently, taking care not to get the solution up along the sides of the flask. Stopper the flask and allow to digest for two hours at 40°C.

After the digestion add approximately 10 glass beads, a piece of paraffin (0.5 to 1 g) and about 0.5 g of finely ground mill feed to the sample in the digestion flask. Then add 10 ml of 25% calcium chloride solution and 10 ml of 10% sodium hydroxide solution. Add sufficient water to make a total volume not to exceed 350 ml and distill 200 ml into 20 ml of standard acid. Back titrate with standard alkali.

A blank determination should be made using 20 ml of distilled water in place of the 20 ml aliquot of sample. One ml of 0.1 *N* acid is equivalent to 0.0030 g of urea.

Samples of both feeds and solutions containing urea in amounts normally added to feeds were tested. Checks were in close agreement, and the percent of error was comparable to that experienced in regular protein determinations of feeds.

Literature Cited

Griem, W. B.
1941 Changes in the Official and Tentative Methods of Analysis made at the fifty-sixth annual meeting, October 28, 29, and 30, 1940. Outline of Method. J. Assoc. Official Agr. Chem. 24: 66–101.

1941a Report on ammoniacal and urea nitrogen (feeds). J. Assoc. Official Agr. Chem. 24: 867–870.
1942 Changes in the Official and Tentative Methods of Analysis—Recommendation of adoption of method as official (first action) with minor changes. J. Assoc. Official Agr. Chem. 25: 92–93.
1942a Report on ammoniacal and urea nitrogen. J. Assoc. Official Agr. Chem. 25:874–877.
1944 Report on ammoniacal urea and nitrogen salts. J. Assoc. Official Agr. Chem. 27: 494–495.

BOOK REVIEWS

Physical Methods of Organic Chemistry. II. Edited by Arnold Weissberger, Eastman Kodak Company. Interscience Publishers, Inc., New York, N. Y. 1946. 631 pp., 15 × 23 cm. Price $8.50.

This second volume of the two-volume series on the subject maintains the excellence of subject matter treatment and of workmanship which has been noted in a review of the first volume (*Cereal Chemistry* 23: 114, 1946). Pages are numbered consecutively in the two volumes and a subject index (52 pp.) covering both volumes concludes the second volume.

Methods applicable to 10 additional types of physical measurements are treated in this volume. The subject matter, authors, and number of pages devoted to the discussion of each subject are as follows: XVII. Spectroscopy and Spectrophotometry—W. West (86 pp.). XVIII. Colorimetry, Photometric Analysis, and Fluorimetry—W. West (46 pp.). XIX. Polarimetry—W. Heller (120 pp.). XX. Determination of Dipole Moments—C. P. Smyth (22 pp.). XXI. Conductometry—T. Shedlovsky (40 pp.). XXII. Potentiometry—L. Michaelis (64 pp.). XXIII. Polarography—O. H. Muller (98 pp.). XXIV. Determination of Magnetic Susceptibility—L. Michaelis (36 pp.). XXV. Determination of Radioactivity—W. F. Bale and J. F. Bonner, Jr. (42 pp.). XXVI. Mass Spectrometry—D. W. Stewart (24 pp.).

The detailed and critical discussions contained in these volumes of the many types of physical measurements already in common use in the chemical laboratory can lead to a more accurate utilization of these methods. Perhaps an even more important function of these volumes will result from the description found therein of several equally important but as yet little used methods. It is especially appropriate that discussions of methods for isotope estimation should be included in these volumes in view of the probability that so much use will be made of these elements in organic and biological studies in the near future.

D. R. Briggs
Division of Agricultural Biochemistry
University of Minnesota
University Farm, St. Paul, Minnesota

Enzymes and Their Role in Wheat Technology. Edited by J. A. Anderson. American Association of Cereal Chemists Monograph Series. Vol. I. ix + 371 pp. Interscience Publishers, Inc., New York, N. Y. 1946. Price $4.50.

The first volume of this new monograph series incorporates an unusual feature: namely, each enzyme class that is reviewed is presented first from the viewpoint of basic enzymology and secondly, in a twin chapter under a different authorship, from the more specific viewpoint of wheat technology. The chapters dealing with basic enzymology are not limited to cereal enzymes but rather present the broad characteristics of the enzyme classes discussed. The classes of enzymes reviewed are properly limited to those of greatest interest to cereal chemists—amylases, proteases, lipases, oxidases, and the fermentation enzymes.

The chapter on the general chemistry of enzymes by Sandstrom has no companion chapter. This condensed chapter will be of interest primarily to those who have little knowledge of enzymology. It is necessarily incomplete.

The first chapter on amylases by Caldwell and Adams reviews higher plant, animal, *Aspergillus oryzae*, and bacterial amylases and (briefly) phosphorylase. The more than 300 references cited were selected to emphasize the varied character and modes of action of the amylases. The companion chapter by Kneen and Sandstedt critically discusses amylases in milling and baking technology. The enzymologist as well as the technologist will find this chapter interesting.

The chapter on esterases by Longenecker concerns chiefly lipolytic esterases. Unexpected in a review of this type but of interest to many is a valuable discussion of methods of measuring esterase activity. The twin chapter by Sullivan relative to esterases (lipases and phosphatases) in milling and baking makes a plea for more fundamental experimental data. The phytase-phytin-calcium system is interestingly reviewed.

An excellent pedagogical discussion of classes or types of oxidizing enzyme systems is given by Barron. The companion chapter by Sullivan concerns the occurrence of oxidizing enzymes in wheat rather than their relation to wheat technology, doubtless because of the dearth of definite information concerning such relation. However, in discussing the oxidation-reduction systems in flour and dough and in discussing lipoxidase the possible relation of enzymes to aging and improving flours is considered.

The stimulating review of proteases by Balls and Kies emphasizes the over-all enzymic characteristics as well as the specificity of the proteases. Proteinases and peptidases of plant, animal, and microbial origin receive attention and serve to illustrate the basic principles encountered in protease research. The companion chapter by Hildebrand concerns the role of proteases in baking. A clear presentation of the controversy concerning the mechanism by which oxidizing agents exert an "improving" action in bread baking is given. It is concluded that the bulk of evidence favors a direct action of oxidizing agents on flour proteins rather than an indirect effect involving enzyme inactivation (or, inversely, activation). Indeed, the possibility that proteases play a part in baking, even including proteases of malted wheat, yeast, and insects, has dwindled to such a minor or at best inconclusive stature that, without meaning any criticism, this chapter might have been entitled, "The Lack of Role of Proteases in Baking." On the other hand, it is unlikely that the controversy is dead.

The last two chapters concern fermentation: "The Mechanism of Alcoholic Fermentation" by Werkman and "Yeast Fermentation" by Atkin, Schultz, and Frey. Knowledge of the 10 to 13 main steps in alcoholic fermentation, which are described clearly by Werkman, prepares the reader in part, at least, for the large measure of control procedures necessary in panary yeast fermentation. Thus, temperature, pH, acidity change during yeast fermentation, sugar concentration and type, phosphate and sulfate, magnesium and potassium, nitrogen type, vitamins, and toxic substances are considered. This chapter and perhaps the chapter on amylases in milling and baking technology will be of greatest interest to technologists.

The contents of the book are derived in the main from literature available before 1944—this reviewer noted only an occasional 1944 and no 1945 references. Fortunately, the basic nature of the information presented minimizes this failing. Misstatements, misprints, and omissions are relatively few and do not significantly lower the quality of the work. The book is a valuable one on basic enzymology as well as a valuable source of information on the role of enzymes in wheat technology. Useful author and subject indexes are included. References are given by title as well as by author.

Hans Lineweaver
Berkeley, California

Currents in Biochemical Research. Edited by D. E. Green. 500 pp. Interscience Publishers, New York, N. Y. 1946. Price $5.00.

The purpose of this book, as aptly stated in the preface, is "an attempt by some thirty research workers to describe in as simple a language as possible the important developments in their own fields and to speculate a little on the most likely paths of future progress." The success of most of the contributors in attaining this objective makes the book both interesting and stimulating and a useful contribution. It is difficult to give in clear and simple language an accurate evaluation of advances in a technical field. Consequently, several of the essays resemble review articles intended for the specialist. The essays cover far too wide a field for the reviewer to attempt an appraisal of the adequacy of their content. However, the reputation of the contributors should insure the authoritative nature of the presentations.

The contents are as follows: 1. G. W. Beadle, The Gene and Biochemistry. 2. W. M. Stanley, Viruses. 3. H. Gaffron, Photosynthesis and the Production of

Organic Matter on Earth. 4. René J. Dubos, The Bacterial Cell. 5. D. R. Hoagland, The Nutrition and Biochemistry of Plants. 6. C. A. Elvehjem, Biological Significance of Vitamins. 7. Karl Folkers, Some Aspects of Vitamin Research. 8. Donald D. Van Slyke, Quantitative Analysis in Biochemistry. 9. Joseph S. Fruton, Enzymic Hydrolysis and Synthesis of Peptide Bonds. 10. Fritz Lipmann, Metabolic Process Patterns. 11. David E. Green, Biochemistry from the Standpoint of Enzymes. 12. Severo Ochoa, Enzymic Mechanisms of Carbon Dioxide Assimilation. 13. B. A. Houssay, Hormones. 14. Leonor Michaelis, Fundamentals of Oxidation and Reduction. 15. Herman M. Kalckar, Mesomeric Concepts in the Biological Sciences. 16. Max A. Lauffer, Viscometry in Biochemical Investigations. 17. D. Rittenberg and David Shemin, Isotope Technique in the Study of Intermediary Metabolism. 18. Karl Meyer, Mucolytic Enzymes. 19. Konrad Bloch, Some Aspects of Intermediary Metabolism. 20. Gregory Pincus, The Steroid Hormones. 21. Kenneth V. Thimann, Plant Hormones and the Analysis of Growth. 22. David Nachmansohn, Chemical Mechanism of Nervous Action. 23. D. W. Woolley, Some Aspects of Biochemical Antagonism. 24. Rollin D. Hotchkiss, Chemotherapy: Applied Cytochemistry. 25. Arnold D. Welch and Ernest Bueding, Biochemical Aspects of Pharmacology. 26. Charles L. Hoagland, Some Biochemical Problems Posed by a Disease of Muscle. 27. C. H. Best, Physiology and Biochemistry. 28. I. Fankuchen and H. Mark, X-Ray Diffraction and the Study of Fibrous Proteins. 29. Michael Heidelberger, Immunochemistry. 30. W. H. Sebrell, Social Aspects of Nutrition. 31. L. C. Dunn, Organization and Support of Science in the United States.

Some variations in the editorial composition are apparent. They range in length from the tart, eight-page contribution by Heidelberger on immunochemistry to the broader twenty-four page discussion by Gaffron on photosynthesis. Some of the articles have only a few selected references while others have much longer bibliographies.

The reviewer was impressed by the manner in which some potentially complex subjects were presented, as in the articles on oxidation and reduction mechanisms and on X-ray studies of protein structure. Many of the essays, such as those of Dubos, Hoagland, Woolley, and Beadle, provoke interesting speculation as to future developments. The essays by Rittenberg and Shemin and by Bloch on intermediary metabolism are both well written but overlap in many respects.

The emphasis of the book is on the increasing store of fundamental biochemical knowledge, although various writers have clearly stated possible application of newer developments. In particular, Sebrell makes a strong plea for more application of nutritional knowledge.

The book is commendably free from typographical errors; only a few were found in a careful reading. The print and binding are satisfactory. The book merits and undoubtedly will have a good reception.

PAUL D. BOYER
Division of Agricultural Biochemistry
University of Minnesota
University Farm, St. Paul, Minnesota

The Chemistry of the Carbon Compounds. Volume III. The Aromatic Compounds. By Victor von Richter. Edited by Richard Anschütz. Translated from Volume II, part 2 of the 12th German edition (compiled by F. Arndt, A. Butenandt, F. Rochussen, R. Tschesche, A. Weissberger) by A. J. Mee. Elsevier Publishing Co., Inc., New York, N. Y. xviii + 794 pp. Price $15.00.

This translation of the well-known text by Victor von Richter is a welcome and worth-while addition to our shelf of reference books on organic chemistry. *The Chemistry of the Carbon Compounds* has appeared in numerous German and English editions and has been a standard reference for many years. The present volume gives an exhaustive treatment of the field of aromatic compounds and maintains the high standards of previous volumes. Theoretical discussions are limited and not extensive and most of the material deals with the general preparations and properties of aromatic compounds.

The book under review is a translation of the second part of the second volume of the twelfth German edition with the exception of the section on organic free radicals which has been shifted to volume IV. Unfortunately, the war has prevented the translators from entirely revising and bringing this book up to date as in the case of previous volumes. The first 50 pages have been revised by Dr. T. W. J. Taylor, but the remainder is a literal translation of the original German text without revisions or additions. Thus the literature is complete to 1936.

The book is divided into two main sections: Part I, Mononuclear Aromatic Compounds, Benzene Derivatives (492 pp.); and Part II, Multinuclear Aromatic Compounds (220 pp.). In the first part there are chapters on the mononuclear benzene hydrocarbons, halogen derivatives, nitrogen derivatives, aromatic compounds of phosphorus, arsenic, antimony, bismuth, boron, and silicon, phenyl metallic derivatives, sulfonic acids, phenols, quinones, phenyl paraffin alcohols and their oxidation products, and mononuclear aromatic compounds with unsaturated side chains. Part II is divided into three major divisions dealing with the phenyl-benzene group, polyphenyl paraffins, and condensed nuclei. There is an excellent subject index of 79 pages but no author index.

The arrangement of the book is good and the use of fine type in the descriptive sections permits the inclusion of more material than is indicated by the 794 pages. It is unfortunate that this treatise could not be brought up to date; nevertheless this volume, together with the others of the series, comprises one of the finest references available in the field of organic chemistry.

SIDNEY E. MILLER
General Mills, Inc.
Research Department
Minneapolis, Minnesota

INDEX TO VOLUME XXIII

AUTHORS

PAGE

Alcock, A. W. (See A. Hofler) 66, 76

Alexander, T. L. (See V. H. Morris). 540

Anderson, J. A. (See K. Hlynka).... 115

—— (See V. G. Martin)........ 568

Arnold, Aaron. Report of the 1945-46 methods of analysis subcommittee on thiamine assay............... 521

Baker, J. C., H. K. Parker, and M. D. Mize. The distribution of water in dough........................ 30

—— **and M. D. Mize.** Gas occlusion during dough mixing............ 39

——, **H. K. Parker, and M. D. Mize.** Supercentrifugates from dough... 16

Baumgarten, Werner, A. N. Mather, and Leonard Stone. Essential amino acid composition of feed materials . 135

Bayfield, E. G., and E. C. Swanson. Effect of yeast, bromate, and fermentation on bread containing soy flour.................... 104

Beckord, O. C. (See R. M. Sandstedt) 548

Bergdoll, M. S. (See D. M. Doty) . 199

Bliss, Laura, and N. M. Naylor. The phosphorylase of waxy maize..... 177

Boyer, P. D. Book review: Currents in biochemical research, ed. by D. E. Green..................... 603

Briant, A. M., and M. R. Hutchins. Influence of ingredients on thiamine retention and quality in baking powder biscuits................. 512

Briggs, D. R. Book review: Physical methods of organic chemistry. I., ed. by Arnold Weissberger....... 114

—— Book review: Physical methods of organic chemistry. II., ed. by Arnold Weissberger.............. 602

—— **and Martin Hanig.** Chemical state of phosphorus in starch as indicated by titration curves on electrodialyzed starches........... 277

Brimhall, B. (See D. W. Hansen) .. 329

Brunson, A. M. (See D. M. Doty).. 199

Carlson, S. C., F. H. Hafner, and J. W. Hayward. Effect of soy flour and nonfat dry milk solids in white bread on the nutritional quality of the protein as measured by three biological methods................. 305

Christensen, C. M. The quantitative determination of molds in flour.... 322

Curtis, J. J., and F. R. Earle. Analyses of double-cross hybrid corn varieties produced on farms....... 88

—— (See F. R. Earle)............. 504

Dickson, A. D. Book review: Methods of analysis of the American Society of Brewing Chemists, ed. by Louis Ehrenfeld.................... 113

Doty, D. M., M. S. Bergdoll, H. A. Nash, and A. M. Brunson. Amino acids in corn grain from several single cross hybrids.............. 199

Earle, F. R., J. J. Curtis, and J. E. Hubbard. Composition of the component parts of the corn kernel . 501

—— (See J. J. Curtis) .. 88

Edgar, J. (See H. Miller)... . 579

Finney, K. F. Loaf volume potentialities, buffering capacity, and other baking properties of soy flour in blends with spring wheat flour 96

—— **and W. T. Yamazaki.** A micromilling technique using the Hobart grinder...... 481

—— **and** ——. Water retention capacity as an index of the loaf volume potentialities and protein quality of hard red winter wheats . 416

Fisher, M. H., and W. O. S. Meredith. Note on a semimicro milling procedure for durum wheat 565

Freilich, Jacob. (See Floyd Schoonover)................ 186

French, C. S. Book review: Frontiers in chemistry. IV. Major instruments and their applications to chemistry, ed. by R. E. Burk and Oliver Grummitt................ 335

Geddes, W. F. Book review: Hackh's chemical dictionary, by Julius Grant 527

—— (See A. Hoffer) 52, 76

—— (See Max Milner)........ 225, 449

—— (See P. P. Noznick).. 297

Glick, David. (See Marjorie Howe).. 360

Hafner, F. H. (See S. C. Carlson)... 305

Hanig, Martin. (See D. R. Briggs).. 277

Hansen, D. W., B. Brimhall, and G. F. Sprague. Relationship of zein to the total protein in corn........... 329

Harris, R. H. (See L. D. Sibbitt).... 559

—— (See G. S. Smith)....... . 471

Hayward, J. W. (See S. C. Carlson). 305

Heider, M. F. (See F. E. Horan).... 492

Hildebrand, F. C. Book review: Official and tentative methods of analysis of the Association of Official Agricultural Chemists, 6th ed. 527

Hlynka, K., and J. A. Anderson. A machine for measuring the extensibility and resistance to extension of gluten......................... 115

Hoffer, A., and A. W. Alcock. A rapid method for the determination of bromate in bromated flours........ 66

——, —— **and W. F. Geddes.** The distribution of thiamine in wheat seedlings at different stages of germination in the dark............ 76

——, **W. F. Geddes, and M. N. Levine.** Effect of leaf and stem rust on the phosphorus metabolism of hard red spring wheat........... 52

Horan, F. E., and M. F. Heider. A study of sorghum and sorghum starches...................... 492

Howe, Marjorie. Further studies on the mechanism of the action of oxidation and reduction on flour...... 84
– – – **and David Glick.** The proteolytic enzyme activity of milled fractions of wheat and the effect of bromate in flour................ 360
Hubbard, J. E. (See F. R. Earle)... 504
Hutchins, M. R. (See A. M. Briant). 512

Irvine, G. N. (See V. G. Martin).... 568

Jesperson, Ethel. (See G. S. Smith). 471
Johnson, J. A., J. A. Shellenberger, and C. O. Swanson. Amylograph curve characteristics of various types of commercial flours and their relation to flour maltose and gassing power values..... 410
——, ——, **and** ——. Extensograph studies of commercial flours and their relation to certain other physical dough tests................. 400
– ——, ——, **and** – ——. Farinograms and mixograms as a means of evaluating flours for specific uses.... ... 388
Jones, C. R., and T. Moran. A study of the mill streams composing 80% extraction flour with particular reference to their nutrient content.. 248
Jordan, Ruth, and M. S. Pettijohn. Use of spray-dried whole-egg powder in sponge cakes. 265

Kik, M. C. Effect of parboiling on thiamine in rice 529
Koehn, R. C. (See A. A. Towner)... 64

Landis, Quick. (See Sutton Redfern) 1
Levine, M. N. (See A. Hoffer). 52
Lineweaver, Hans. Book review: Enzymes and their role in wheat technology, ed. by J. A. Anderson..... 602

Martin, V. G., G. N. Irvine, and J. A. Anderson. A micro method for making macaroni........... .. 568
Mather, A. N. (See Werner Baumgarten)... 135
Meredith, W. O. S. Analysis of data for A.A.C.C. check sample service. II. Protein and thiamine results, 1944–45....... 585
—— (See M. H. Fisher) 565
Merritt, P. P. (See P. P. Noznick).. 297
Miller, H., J. Edgar, and A. G. O. Whiteside. Effect of gassing rate on loaf volume....... 579
Miller, S. E. Book review: The chemistry of the carbon compounds. Volume III. The aromatic compounds, by Victor von Richter... 604
—— Book review: Organic preparations, by Conrad Weygand....... 336
Milner, Max, and W. F. Geddes. Grain storage studies. III. The relation between moisture content, mold growth, and respiration of soybeans......................... 225
—— **and** ——. Grain storage studies. IV. Biological and chemical factors involved in the spontaneous heating of soybeans.. 449
Mize, M. D. (See J. C. Baker). 16, 30, 39
Moran, T. (See C. R. Jones)...... 248
Morris, V. H., T. L. Alexander, and E. D. Pascoe. Studies of the composition of the wheat kernel. III. Distribution of ash and protein in central and peripheral zones of whole kernels........ 540

Nash, H. A. (See D. M. Doty)..... 199
Naylor, N. M. (See Laura Bliss).... 177
Noznick, P. P., P. P. Merritt, and W. F. Geddes. Staling studies on breads containing waxy maize starch 297

Parker, H. K. (See J. C. Baker).. 16, 30
Pascoe, E. D. (See V. H. Morris)... 540
Pettijohn, M. S. (See Ruth Jordan). 265
Pfeiffer, S. E. (See J. M. Van Laren) 428
Price, S. A. The vitamin B-complex content of the commercial milling products of Egyptian rice........ 318

Redfern, Sutton, and Quick Landis. Methods for determination of alpha-amylase. II. Liquefying and dextrinizing activities of alpha-amylases from different sources........... 1
– — (See Floyd Schoonover) 186
Richards, W. A. Book review: The baking industry, by Mary Kinnavey Moore 528
—— (See Betty Sullivan).......... 365

Sandstedt, R. M. Photomicrographic studies of wheat starch. I. Development of the starch granules.... 337
—— **and O. C. Beckord.** Photomicrographic studies of wheat starch. II. Amylolytic enzymes and the amylase inhibitor of the developing wheat kernel................. ... 548
Schmalz, F. D. (See Margaret Schmoll)... 600
Schmoll, Margaret, and F. D. Schmalz. Determination of urea and ammoniacal nitrogen in feeds 600
Schoonover, Floyd, Jacob Freilich, and Sutton Redfern. Some effects of cabinet fermentation on sponge temperatures and dough and bread characteristics............. 186
Schwain, F. R. Report of the 1945–1946 committee on testing biscuit and cracker flours.. 440
Shellenberger, J. A. (See J. A. Johnson)............... ... 388, 400, 410
Sibbitt, L. D., and R. H. Harris. Some observations on the visual color of tubular and disc macaroni.. 559
—— (See G. S. Smith)............ 471
Smiley, W. G., and A. K. Smith. Preparation and nitrogen content of soybean protein................. 288
Smith, A. K. (See W. G. Smiley).... 288
Smith, G. S., R. H. Harris, Ethel Jesperson, and L. D. Sibbitt. The effect of pressure on macaroni discs: size and number of air bubbles in relation to light transmission....... 471
Sprague, G. F. (See D. W. Hansen). 329
Stone, Leonard. (See Werner Baumgarten)...................... 135

Sullivan, Betty. Book review: Studies on the nature of the bromate effect, by Holger Jørgensen.............. 446
—— **and W. A. Richards.** Open trough and cabinet fermentation of bread sponges.................... 365
Swanson, C. O. (See J. A. Johnson) 388, 400, 410
—— (See E. C. Swanson).... 432, 590
Swanson, E. C., and C. O. Swanson. Gas production and gas retention of dough as affected by type of flour, baking formula, and amount of mixing........................ 432
—— **and** ——. Modification of the gas production and gas retention properties of dough by some surface-active, reducing, and oxidizing agents........................ 590
—— (See E. G. Bayfield)............ 104
—— (See Earl B. Working).......... 210

Tanner, F. W., Jr. (See J. M. Van Lanen).......................... 428
Tinklin, G. L., and G. E. Vail. Effect of method of combining the ingredients upon the quality of the finished cake.................... 155
Towner, A. A., and R. C. Koehn. Rapid determination of bromate in flour.............................. 64
Tucker, I. W. Effect of flour lipids on recovery of gluten from hard and soft wheat flours by the use of sulfite solutions........................ 217

Vail, G. E. (See G. L. Tinklin)...... 155
Van Lanen, J. M., F. W. Tanner, Jr., and S. E. Pfeiffer. Composition of hybrid corn tassels.............. 428

Watson, H. A. Study of some factors influencing the oxidation of thiamine to thiochrome.................. 166
Whiteside, A. G. O. (See H. Miller) 579
Working, E. B., and E. C. Swanson. An automatic device for the measurement of gas production and gas retention in doughs............... 210

Yamazaki, W. T. (See K. F. Finney) 416, 484

SUBJECTS

PAGE

- **A.A.C.C.,** see American Association of Cereal Chemists
- **Alpha-amylase,** see Amylases
- **American Association of Cereal Chemists**
  - Check sample service on protein and thiamine (Meredith)............ 585
  - Committee reports, see Committee reports
- **Amino acids**
  - Microbiological assay of feeds (Baumgarten, Mather, and Stone) 135
  - Of feed materials (Baumgarten, Mather, and Stone)......... ... 135
  - Of hybrid corn grains (Doty, Bergdoll, Nash, and Brunson)....... 199
- **Amylases**
  - Activities of alpha-amylases from different sources (Redfern and Landis)...................... 1
  - Bacteria, determination by liquefying and dextrinizing activity (Redfern and Landis).............. 1
  - Barley, determination by liquefying and dextrinizing activity (Redfern and Landis).................. 1
  - Determination (Redfern and Landis) 1
  - Inhibitor, in developing wheat kernel (Sandstedt and Beckord). ... 548
  - Liquefying and dextrinizing activities (Redfern and Landis)....... 1
  - Mold, determination by liquefying and dextrinizing activity (Redfern and Landis).................. 1
  - Wheat, of developing kernel (Sandstedt and Beckord)............ 548
- **Amylograms**
  - Of commercial flours (Johnson, Shellenberger, and Swanson)..... 410
  - Relation to flour maltose and gassing power values (Johnson, Shellenberger, and Swanson).......... 410
- **Amylograph curves,** see Amylograms
- **Ash,** In wheat, distribution in central and peripheral zones of whole kernel (Morris, Alexander, and Pascoe)... 540

- **Baking**
  - Formulae, effect on gas production and retention (Swanson and Swanson)........................ 432
  - Quality, see Flour, Baking quality
- **Biscuit flour**
  - Evaluation by means of
    - amylograms (Johnson, Shellenberger, and Swanson)......... 410
    - extensograms (Johnson, Shellenberger, and Swanson)......... 400
    - farinograms (Johnson, Shellenberger, and Swanson)......... 388
    - mixograms (Johnson, Shellenberger, and Swanson)......... 388
- **Biscuits,** Baking powder
  - Ingredients, effect on quality (Briant and Hutchins)............... 512
  - Thiamine retention as affected by ingredients (Briant and Hutchins) 512

PAGE

- **Book reviews**
  - The baking industry, by Mary Kinnavey Moore (Richards)........ 528
  - The chemistry of the carbon compounds. III. The aromatic compounds, by Victor von Richter (Miller)...................... 604
  - Currents in biochemical research, ed. by D. E. Green (Boyer)...... 603
  - Enzymes and their role in wheat technology, ed. by J. A. Anderson (Lineweaver)................. 602
  - Frontiers in chemistry. IV. Major instruments and their applications to chemistry, ed. by R. E. Burk and Oliver Grummitt (French).. 335
  - Hackh's chemical dictionary, by Julius Grant (Geddes).......... 527
  - Methods of analysis of the American Society of Brewing Chemists, ed. by Louis Ehrenfeld (Dickson).... 113
  - Official and tentative methods of analysis of the Association of Official Agricultural Chemists, 6th ed. (Hildebrand)................. 527
  - Organic preparations, by Conrad Weygand (Miller).............. 336
  - Physical methods of organic chemistry. I., ed. by Arnold Weissberger (Briggs)............... 114
  - —— II., ed. by Arnold Weissberger (Briggs)..................... 602
  - Studies on the nature of the bromate effect, by Holger Jørgensen (Sullivan)........................ 446
- **Bread**
  - Baking properties of soy flour in blends with spring wheat flour (Finney).................... 96
  - Containing soy flour, effect of yeast, bromate, and fermentation (Bayfield and Swanson)............ 104
  - Effect of cabinet fermentation (Schoonover, Freilich, and Redfern)......................... 186
  - Flour, see Flour
  - Milk solids in (Carlson, Hafner, and Hayward).................... 305
  - Nutritive value
    - milk solids for improving (Carlson, Hafner, and Hayward).... 305
    - soy flour for improving (Carlson, Hafner, and Hayward)........ 305
  - Quality, effect of cabinet fermentation (Schoonover, Freilich, and Redfern).................... 186
  - Soybean flour in
    - (Bayfield and Swanson)......... 104
    - (Carlson, Hafner, and Hayward). 305
  - Sponges, see Dough, Sponge
  - Staling, effect of starch (Noznick, Merritt, and Geddes)........... 297
- **Bromate**
  - Effect on
    - bread containing soy flour (Bayfield and Swanson)........... 104
    - protease activity in flour (Howe and Glick)................ 360

Bromate (Continued)
In flour
determination
(Towner and Koehn).... 64
(Hofler and Alcock)....... 66
extraction with carbon tetrachloride (Towner and Koehn). 61

Cabinet fermentation, see Fermentation, Cabinet
Cake
Baking tests, effect of methods of mixing ingredients (Tinklin and Vail)........ 155
Effect of method of combining ingredients on quality (Tinklin and Vail)........ 155
Flour, evaluation by means of
amylograms (Johnson, Shellenberger, Swanson)........ 410
extensograms (Johnson, Shellenberger, and Swanson)......... 400
farinograms (Johnson, Shellenberger, and Swanson)....... 388
mixograms (Johnson, Shellenberger, and Swanson)......... 388
Ingredients, effect of method of combining on quality (Tinklin and Vail)........ 155
Sponge, made with spray-dried whole-egg-powder (Jordan and Pettijohn)........ 265
Cereals and cereal products
Storage
biological and chemical factors involved in spontaneous heating (Milner and Geddes)......... 449
effect of moisture content on mold growth and respiration (Milner and Geddes)........ 225
Committee reports
Committee on testing biscuit and cracker flours, 1945–46 (Schwain). 440
Subcommittee on thiamine assay, 1945–46 (Arnold)........ 521
Corn (see also Corn, Hybrid)
Amino acid content of single cross hybrids (Doty, Bergdoll, Nash, and Brunson)........ 199
Composition of parts of kernel (Earle, Curtis, and Hubbard).... 504
Hybrid
amino acid content of single cross varieties (Doty, Bergdoll, Nash, and Brunson)........ 199
ash content of double-cross varieties (Curtis and Earle)........ 88
oil content of double-cross varieties (Curtis and Earle)........ 88
protein content of double-cross varieties (Curtis and Earle).... 88
protein content of single cross varieties (Doty, Bergdoll, Nash, and Brunson)........ 199
starch content of double-cross varieties (Curtis and Earle).... 88
sugar content of double-cross varieties (Curtis and Earle).... 88
tassels, composition (Van Lanen, Tanner, and Pfeiffer)......... 428
varieties, chemical composition (Curtis and Earle)........ 88

Corn (Continued)
Oil
content of hybrid varieties (Curtis and Earle)........ 88
Protein, zein content (Hansen, Brimhall, and Sprague)........ 329
Starch, see Starch, Corn
Sugar, content of hybrid varieties (Curtis and Earle)........ 88
Tassels, composition of hybrid varieties (Van Lanen, Tanner, and Pfeiffer)........ 428
Waxy maize, phosphorylase of (Bliss and Naylor)........ 177
Cracker flour
Evaluation by means of
amylograms (Johnson, Shellenberger, and Swanson)......... 410
extensograms (Johnson, Shellenberger, and Swanson)......... 400
farinograms (Johnson, Shellenberger, and Swanson). 388
mixograms (Johnson, Shellenberger, and Swanson). 388
Testing (Schwain)........ 410

Dough
Density, method for measuring (Baker and Mize).... ... 39
Effect of protease inhibitors on quality (Howe)........ 84
Gas occlusion during mixing, measured by density measurements (Baker and Mize)........ 39
Gas production in, see Gas production in dough
Gas retention in, see Gas retention in dough
Liquid phase (Baker, Parker, and Mize)........ 16
Mixing
curves, see Mixograms
effect on gas retention (Swanson and Swanson)........ 432
gas occlusion during (Baker and Mize)........ 39
Oxidizing agents, effect on gas production and retention (Swanson and Swanson)........ 590
Physical properties
extensograph measurements (Johnson, Shellenberger, and Swanson)........ 400
farinograph and mixograph measurements (Johnson, Shellenberger, and Swanson)........ 388
mixograph measurements (Johnson, Shellenberger, and Swanson)........ 388
Reducing agents, effect on gas production and retention (Swanson and Swanson)........ 590
Sponge, Fermentation, see Fermentation
Supercentrifugates from (Baker, Parker, and Mize)........ 16
Surface-active agents, effect on gas production and retention) Swanson and Swanson)........ 590
Temperature, effect of cabinet fermentation on (Schoonover, Freilich, and Redfern)........ 186

Dough (*Continued*)
  Testing, comparison of farinogram, mixogram, and extensogram characteristics (Johnson, Shellenberger, and Swanson)........ 400
  Water
    distribution in (Baker, Parker, and Mize)............... 30
    free and bound in (Baker, Parker, and Mize)............... 30
Dried eggs, see Egg powder

Egg powder
  Leavening properties (Jordan and Pettijohn)................... 265
  Spray-dried whole-egg in sponge cake (Jordan and Pettijohn)..... 265
Enzymes
  Assay methods, comparison of liquefying and dextrinizing activity (Redfern and Landis) 1
Extensibility of gluten, Stretchometer for measuring (Hlynka and Anderson)......................... 115
Extensograms
  Effect of cabinet and open trough fermentation (Sullivan and Richards)....................... 365
  For evaluating flours for specific uses (Johnson, Shellenberger, and Swanson)................. 400
  Of commercial flours, compared with other physical dough tests (Johnson, Shellenberger, and Swanson). 400
Extensograph curves, see Extensograms

Farinograms
  Effect of
    cabinet fermentation
      (Schoonover, Freilich, and Redfern).................... 180
      (Sullivan and Richards)....... 365
    open trough fermentation (Sullivan and Richards).......... 365
    protease inhibitors (Howe)...... 84
  For evaluating flours for specific uses (Johnson, Shellenberger, and Swanson)................... 388
Farinograph curves, see Farinograms
Feeds
  Amino acid composition (Baumgarten, Mather, and Stone)...... 135
  Ammoniacal nitrogen in (Schmoll and Schmalz)................ 600
  Corn tassels (hybrid) as adjunct (Van Lanen, Tanner, and Pfeiffer) 428
  Microbiological assay of amino acids (Baumgarten, Mather, and Stone) 135
  Nitrogen (ammoniacal) in (Schmoll and Schmalz)................ 600
  Urea and ammoniacal nitrogen in, determination (Schmoll and Schmalz).................... 600
Fermentation
  Cabinet
    as compared with open trough fermentation (Sullivan and Richards)...................... 365
    effects on bread characteristics (Schoonover, Freilich, and Redfern)................... 180

Fermentation (*Continued*)
  Effect on bread containing soy flour (Bayfield and Swanson) . . 104
  Open trough, as compared with cabinet fermentation (Sullivan and Richards)............ .. 365
Flour
  Amylograms of commercial flours (Johnson, Shellenberger, and Swanson)................ 410
  Baking quality, with blends of soy flour (Finney)................ 96
  Biscuit, see Biscuit flour
  Bromated
    assay, see Bromate in flour, determination
    extraction of bromate with carbon tetrachloride (Towner and Koehn).. 64
  Cake, see Cake flour
  Cracker, see Cracker flour
  Diastatic activity, interpreted by amylograms (Johnson, Shellenberger, and Swanson). 410
  Effect of type on gas production and retention (Swanson and Swanson) 132
  Eighty percent extraction, nutritive value (Jones and Moran)... .. 218
  Evaluation for specific uses by means of
    amylograms (Johnson, Shellenberger, and Swanson)......... 410
    extensograms (Johnson, Shellenberger, and Swanson).. 400
    farinograms (Johnson, Shellenberger, and Swanson) 388
    mixograms (Johnson, Shellenberger, and Swanson)......... 388
  Extensograms of commercial flours (Johnson, Shellenberger, and Swanson)............... 400
  Farinograms of commercial flours (Johnson, Shellenberger, and Swanson)... 388
  Lipids, see Lipids in flour
  Maltose value, see Flour, Diastatic activity
  Mechanism of action of oxidation and reduction (Howe)... 81
  Micro-milling technique with the Hobart grinder (Finney and Yamazaki).................... 481
  Mill streams, see Wheat, Milling fractions
  Mixograms of commercial flours (Johnson, Shellenberger, and Swanson).................... 388
  Mold, quantitative determination (Christensen)................. 322
  Nutritive value of 80% extraction (Jones and Moran)............. 248
  Pastry, see Pastry flour
  Protease
    activity, effect of bromate (Howe and Glick)........... . .. 360
    inhibitors, effect on dough quality (Howe).................... 84
  Protein
    content, A.A.C.C. check sample service (Meredith)..... 585
    quality, as expressed by water retention capacity (Finney and Yamazaki)... 416

***Flour* (*Continued*)**
- Semimicro-milling procedure for Durum wheat (Fisher and Meredith) 565
- Soybean, see Soybean flour
- Soluble constituents (Baker, Parker, and Mize) 16
- Thiamine content, A.A.C.C. check sample service (Meredith) 585

Gas production in dough
- Apparatus for measuring (Working and Swanson) 210
- Effect of
  - flour, baking formula, and amount of mixing (Swanson and Swanson) 432
  - oxidizing agents (Swanson and Swanson) 590
  - reducing agents (Swanson and Swanson) 590
  - surface active agents (Swanson and Swanson) 590
  - "Inherent" rates compared with "supplemented" rates (Miller, Edgar, and Whiteside) 579
- Loaf volume affected by (Miller, Edgar, and Whiteside) 579

Gas retention in dough
- Apparatus for measuring (Working and Swanson) 210
- Effect of flour, baking formula and amount of mixing (Swanson and Swanson) 432

Gasometer, Apparatus for measuring gas production and retention in doughs (Working and Swanson) 210

Gluten
- Effect of lipids on recovery (Tucker) 217
- Extensibility
  - measuring machine (Hlynka and Anderson) 115
  - resistance to, measurement (Hlynka and Anderson) 115
- Quality, measurement with Stretchometer (Hlynka and Anderson) 115
- Testing device, Stretchometer (Hlynka and Anderson) 115
- Water-binding action in dough (Baker, Parker, and Mize) 30

Glycinin, In soybeans, purity (Smiley and Smith) 288

Grain, see Cereals and cereal products

Heating, Spontaneous, see Spontaneous heating

Hobart grinder, use in micro-milling technique (Finney and Yamazaki) 484

Hybrid corn, see Corn, Hybrid

Leavening property, of spray-dried whole-egg powder (Jordan and Pettijohn) 265

Lipids, In flour, effect on gluten recovery (Tucker) 217

Loaf volume
- Effect of
  - gassing rate (Miller, Edgar, and Whiteside) 579
  - soy flour additions to wheat flour (Finney) 96
- Water retention capacity as index (Finney and Yamazaki) 416

Macaroni
- Apparatus for making 50 gram sample lots (Martin, Irvine, and Anderson) 568
- Color
  - effect of degree and duration of pressure (Sibbitt and Harris) 559
  - scores, comparison of tubular and disc (Sibbitt and Harris) 559
- Effect of pressure
  - on color (Sibbitt and Harris) 559
  - on air bubbles in discs (Smith, Harris, Jesperson, and Sibbitt) 471
- Light transmission, relation to air bubbles (Smith, Harris, Jesperson, and Sibbitt) 471
- Micro method for making (Martin, Irvine, and Anderson) 568

Maize, see Corn, Waxy maize

Microbiological assay of amino acids in feeds (Baumgarten, Mather, and Stone) 135

Milk solids, used as bread ingredient (Carlson, Hafner, and Hayward) 305

Milling
- Small samples of wheat
  - (Finney and Yamazaki) 484
  - (Fisher and Meredith) 565
- Semimicro procedure for wheat (Fisher and Meredith) 565

Milling fractions, see Wheat, Milling fractions

Mill streams, see Wheat, Milling fractions

Mixing
- Of dough, see Dough, Mixing
- Of ingredients, effect of method on cake quality (Tinklin and Vail) 155

Mixograms
- Effect of
  - density (Baker and Mize) 39
  - occluded gas (Baker and Mize) 39
- For evaluating flours for specific uses (Johnson, Shellenberger, and Swanson) 388

Mixograph curves, see Mixograms

Moisture in soybeans, effect on respiration (Milner and Geddes) 225

Mold growth, effect on respiration of soybeans during storage (Milner and Geddes) 225

Mold in flour, quantitative determination (Christensen) 322

Nicotinic acid in Egyptian rice (Price) 318

Nitrogen
- In feeds, determination of urea and ammoniacal (Schmoll and Schmalz) 600
- In soybean protein (Smiley and Smith) 288

Nutritive value
- Bread, improving with dried milk solids and soy flour (Carlson, Hafner, and Hayward) 305
- Flour, 80% extraction (Jones and Moran) 248
- Milling fractions composing 80% extraction flour (Jones and Moran) 248
- Of corn protein as measured by zein content (Hansen, Brimhall, and Sprague) 329
- Of hybrid corn tassels (Van Lanen, Tanner, and Pfeiffer) 428

Open trough fermentation, see Fermentation, Open trough
Oxidizing agents, Mechanism of action on flour (Howe) 84

Pastry flour
  Evaluation by means of
    extensograms (Johnson, Shellenberger, and Swanson)........ 400
    mixograms (Johnson, Shellenberger, and Swanson)........ 388
Phosphorus in starch, nature of (Briggs and Hanig).................... 277
Phosphorylases in corn, role in formation of starch (Bliss and Naylor). 177
Photomicrographs
  Of developing wheat starch granules
    (Sandstedt)................... 337
    (Sandstedt and Beckord)........ 548
Polysaccharides, see Starch
Potato starch, see Starch, Potato
Proteases
  Activity of milled fractions of wheat (Howe and Glick).............. 360
  In flour, effect of bromate (Howe and Glick)................... 360
  Inhibitors, effect on dough quality (Howe)...................... 84
Proteins (see also individual proteins)
  Composition in hybrid corn (Doty, Bergdoll, Nash, and Brunson) .. 199
  In corn
    hybrid varieties
      (Curtis and Earle)........... 88
      (Doty, Bergdoll, Nash, and Brunson)................ 199
    zein content as measure of nutritional value (Hansen, Brimhall, and Sprague)............... 329
  In flour
    A.A.C.C. check sample service (Meredith)................. 585
    quality measured by water retention capacity (Finney and Yamazaki)................. 416
  In soybeans, effect of extraction method on yield (Smiley and Smith)...................... 288
  In wheat, distribution in central and peripheral zones of whole kernel (Morris, Alexander, and Pascoe). 540
Proteolytic enzymes, see Proteases
Pyridoxine in Egyptian rice (Price)... 318

Reducing agents, Mechanism of action on flour (Howe)............ 84
Respiration of soybeans, as affected by moisture and mold growth during storage (Milner and Geddes)..... 225
Riboflavin in Egyptian rice (Price)... 318
Rice
  Egyptian, vitamin B-complex (Price) 318
  Thiamine content, effect of different methods of parboiling (Kik)..... 529
  Vitamin B-complex of Egyptian (Price)...................... 318
Rust of wheat, see Wheat, Rust

Sorghum
  Grains (Horan and Heider)........ 492
  Starch, see Starch, Sorghum

Soybean
  Flour
    as bread ingredient
      (Carlson, Hafner, and Hayward)...................... 305
      (Finney)... 96
    baking quality in blends with spring wheat flour (Finney) ... 96
    effect in breadmaking (Bayfield and Swanson)..... 104
  Moisture in, effect on respiration (Milner and Geddes)........... 225
  Mold growth, effect on respiration (Milner and Geddes)........... 225
  Protein, nitrogen content (Smiley and Smith).................. 288
  Respiration during storage as related to moisture content and mold growth (Milner and Geddes)..... 225
  Storage
    biological and chemical factors involved in spontaneous heating (Milner and Geddes)......... 449
    effect of moisture content on mold growth and respiration (Milner and Geddes)................ 225
Sponge
  Cake, see Cake, Sponge
  Doughs, see Dough, Sponge
Spontaneous heating of soybeans, biological and chemical factors involved (Milner and Geddes)..... 449
Starch
  Corn (see also Starch, Waxy maize)
    content of hybrid varieties (Curtis and Earle).................. 88
    phosphorus in, chemical nature (Briggs and Hanig)........... 277
    role of phosphorylase in formation (Bliss and Naylor)........... 177
  Electrodialyzed, titration curves as a means of studying phosphorus (Briggs and Hanig)............. 277
  Formation from glucose-1-phosphate by action of phosphorylase (Bliss and Naylor).................. 177
  Phosphorus in (Briggs and Hanig).. 277
  Potato, phosphorus in, chemical nature (Briggs and Hanig)....... 277
  Sorghum, study on (Horan and Heider)...................... 492
  Titration curves in determining nature of phosphorus (Briggs and Hanig)..................... 277
  Waxy maize
    its effect on bread staling (Noznick, Merritt, and Geddes).... 297
    phosphorylase (Bliss and Naylor) 177
  Wheat
    amylases and amylase inhibitor of the developing kernel (Sandstedt and Beckord)........... 548
    development of granule in kernel (Sandstedt)................. 337
    effect on bread staling (Noznick, Merritt, and Geddes)......... 297
    phosphorus in, chemical nature (Briggs and Hanig).......... 277
    photomicrographic studies
      (Sandstedt)............. ... 337
      (Sandstedt and Beckord)...... 548
    water-binding action in dough (Baker, Parker, and Mize).... 30

Storage of soybeans, effect of moisture content on mold growth and respiration (Milner and Geddes) 225
Stretchometer for measuring gluten extensibility (Hlynka and Anderson) 115

Thiamine
  Assay
    by ferricyanide oxidation to thiochrome (Watson)......... ... 166
    by fluorometric analysis (Arnold). 521
    in flour, extraction method for fluorometric assay (Arnold)... 521
  In baking powder biscuits, effect of ingredients on retention (Briant and Hutchins).. 512
  In flour, A.A.C.C. check sample service (Meredith)............ 585
  In rice
    effect of soaking rough rice on milled product (Kik).... 529
    parboiled by different methods (Kik)..................... 520
  In wheat seedlings
    at different stages of germination (Hoffer, Alcock, and Geddes).. 76
    distribution in organs during germination (Hoffer, Alcock, and Geddes)............... 76
  Oxidation by ferricyanide to thiochrome (Watson)............. 166
  Thiochrome assay, factors influencing (Watson)................ 166
Thiochrome assay for thiamine, see Thiamine, Thiochrome assay

Urea in feeds, determination (Schmoll and Schmalz)................. 600

Vitamin B$_1$, see Thiamine
Vitamin B$_2$, see Riboflavin
Vitamin B$_5$, see Nicotinic acid
Vitamin B$_6$, see Pyridoxine
Vitamin B-complex in Egyptian rice (Price)....................... 318

Water retention capacity as an index of loaf volume and protein quality of hard red winter wheats (Finney and Yamazaki)............... 416
Wheat
  Ash, distribution in central and peripheral zones of whole kernel (Morris, Alexander, and Pascoe) 540
  Composition of kernel (Morris, Alexander, and Pascoe)............ 540

Wheat (*Continued*)
  Durum, Semimicro milling procedure for testing (Fisher and Meredith) 568
  Germinating, thiamine content at different stages (Hoffer, Alcock, and Geddes).. . . . 76
  Hard, effect of flour lipids on gluten recovery (Tucker) 217
  Lipids, effect on gluten recovery (Tucker) 217
  Micromilling and testing (Finney and Yamazaki) 181
  Milling, see Milling
  Milling fractions composing 80%, extraction flour nutrient content (Jones and M[illegible]m) 218
  Phosphorus content, effect of leaf and stem rust (Hoffer, Geddes, and Levine) 52
  Protein, distribution in central and peripheral zones of the whole kernel (Morris, Alexander, and Pascoe).. 540
  Proteases activity in milled fractions (Howe and Glick) . . . 30[illegible]
  Rust, effect on phosphorus metabolism of hard red spring (Hoffer, Geddes, and Levine).. 52
  Soft, effect of flour lipids on gluten recovery (Tucker)........... 217
  Spring, hard
    baking qualities of blends with soy flour (Finney).......... 96
    effect of leaf and stem rust on phosphorus metabolism (Hoffer, Geddes, and Levine).......... 52
  Starch, see Starch, Wheat
  Testing, Semimicro milling procedure (Fisher and Meredith).... 568
  Thiamine, distribution in organs during germination (Hoffer, Alcock, and Geddes)............. 76
  Varieties, Semimicro milling procedure for testing (Fisher and Meredith)............... 568
  Winter, hard, Water retention capacity as an index to loaf volume protein quality (Finney and Yamazaki).. 416

Yeast, effect on bread containing soy flour (Bayfield and Swanson) . 104

Zein, relation to total corn protein (Hansen, Brimhall, and Sprague) 329